RESISTANCE TO *Xanthomonas campestris* pv. *vesicatoria* IN TOMATO

BY

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KEY TO ABBREVIATIONS

Name	Abbreviation
Aconitase	Aco
Alcohol dehydrogenase	Adh
Acid phosphatase	Aps
ß-N-Acetyl glucosaminidase	Bnag
Diaphorase	Dia
Esterase	Est
Formate dehydrogenase	Fdh
Glutamate oxaloacetate transaminase	Got
Nitrate reductase	Nir
6-Phosphogluconate dehydrogenase	6Pgdh
Phosphoglucose isomerase	Pgi
Phosphoglucomutase	Pgm
Punctate	Pn
Peroxidase	Prx
Shikimate dehydrogenase	Skdh
Superoxide dismutase	Sod
Triose phosphate isomerase	Tpi

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The resistance in an accession of Lycopersicon esculentum Mill., Hawaii 7998 (H7998), to Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv) is associated with a hypersensitive reaction (HR). The reaction in the resistant line changed to a susceptible reaction when the incubation temperature increased from 24 degrees C to 30 degrees C after inoculation with strains of race 1 of the tomato group of Xcv (XcvT). A cross was made between H7998 and a susceptible tomato breeding line Fla. 7060. The phenotype of HR in the F_1 plants was intermediate based on the time for confluent necrosis and bacterial growth curves. Continuous variation in time for necrosis after inoculation was observed in the F_2 population. Therefore, the hypersensitive resistance in H7998 is inherited as a quantitative trait, which is unique in plant-microbe interactions.

An Xcv strain (XV56) from Brazil that is compatible in H7998 was used as a recipient for the cloned avirulence gene, avrRxv, which is necessary for HR in H7998. The development of necrosis induced by this transconjugant and a wild strain of race 1 of XcvT (90-14) was compared in F_2 plants. A possibility of the existence of other avirulence genes in 90-14 interacting with H7998 was suggested by the dynamics of the development of necrosis.

The quantitative inheritance of the HR in H7998 to strain 90-14 was further demonstrated by studying the linkage relationships between genes associated with the hypersensitive resistance in H7998 and isozyme loci. A line of L. pennellii, LA 716, was used as the susceptible parent in crosses because of its great genetic diversity compared to H7998. In the progeny of a backcross of F_1 plants to H7998, 6 linkages were detected. The isozymes linked to HR were Prx-1 and Skdh-1 on chromosome 1, Pgm-2 on chromosome 4, Got-2 on chromosome 7, Sod-1 on chromosome 11, and a morphological marker, Pn, on chromosome 8.

A strain of Xcv from Brazil (XV56) which can cause a susceptible reaction in the plant of H7998 was identified as a new race of XcvT (race 2). Over 200 tomato accessions were screened for resistance to this race. A slow HR to race 2 was observed in plants of PI155372 and PI79532. Fewer lesion numbers and smaller lesion sizes were observed in the plants of PI114490, PI128216, and PI306216.

CHAPTER 1
INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is one of the most important vegetable crop produced in the USA. Fresh market tomato is planted in 182,000 ha with a production of 7,000,000 tons in the USA (Tigchelaar, 1986). In Florida, tomato production is economically the most important vegetable crop. The total economic value represented 28.7% of all vegetables produced in the state, or more than four times that of any other vegetable (Pohronezny et al., 1986). Potentially the most harmful disease of tomato is bacterial spot caused by Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv). The disease affects production, occasionally resulting in heavy economic losses. In some fields 50-70% of foliage may be lost (Pohronezny et al., 1986). Experiments including inoculation of field plots have demonstrated the adverse impact of bacterial spot on tomato yield, fruit quality, and size (Pohronezny and Volin, 1983).

The climate in Florida is subtropical with predominantly mild temperatures and seasonal periods of high rainfall. The cropping patterns in southern Florida are reversed from those in temperate regions with the noncropping season in the hot, humid months of June, July and August (Pohronezny et al.,

1986). The pathogen is endemic in Florida and many other subtropical regions of the world. Survival of the bacterium from one season to another, in Florida, is mainly in tomato volunteers and crop residues (Jones et al., 1986). Survival in other areas may be on alternate hosts (Srinivasan et al., 1962; Dye et al., 1964) or on stalks of dead tomato plants (Peterson, 1963; Goode and Sasser, 1980). Thus, exclusion of the bacterium to prevent the occurrence of the disease is difficult.

Traditionally, foliar application of chemicals, such as streptomycin and fixed copper compounds, has been used to control the disease. In the late 1950s, streptomycin provided good control of bacterial spot. Within a short time, this antibiotic failed to control the disease due to the development of a resistant population of the bacterium (Thayer and Stall, 1962). Although copper compounds have been used for a long time to control the disease, copper resistant strains of Xcv have been detected in those areas (Marco and Stall, 1983).

The inefficiency of chemical and cultural control measures in controlling bacterial spot makes resistance breeding an attractive approach. Several researchers have reported genotypes carrying some level of resistance to bacterial spot (Coyne and Schuster, 1967; Crill et al., 1972; Volin, 1979; Lawson and Summers, 1984). However, only recently a high level of field resistance was discovered in a bacterial wilt-resistant accession, Hawaii 7998 (Scott and

Jones, 1986). This field resistance is controlled by quantitative gene action (Scott and Jones, 1989). The phenotype of F_1 plants is intermediate and the F_2 population has a continuous distribution. Thus, a modified backcross method has been suggested in a breeding program with screening of the F_2 and F_3 to obtain homozygosity of resistance before making the next backcross (Scott and Jones, 1989). Potentially, screening for resistance could be accomplished by screening for a hypersensitive reaction (HR) to Xcv which was demonstrated in this accession. However, the relationship of HR and field resistance is not clear. Hypersensitivity is not usually associated with polygenic inheritance. Therefore, the purpose of this dissertation was to study the linkages of HR intensity and isozyme markers. This was done to determine if HR is affected by several genes in tomato.

The Xcv strains in Florida and other areas of the United States cause HR in the plants of Hawaii 7998 (Stall, personal communication). However, this resistant line was reported to be susceptible to the local strains of Xcv in Brazil (Nagai and Sugimori, 1986). The Brazilian strain was examined further to determine whether it was a new race of Xcv based on the reactions of differential host plants and the ability to induce HR in Hawaii 7998. A collection of tomato genotypes was used to screen for resistance to the Brazilian strain.

CHAPTER 2
LITERATURE REVIEW

Isozymes in Breeding and Genetics

Isozymes are multiple molecular forms of enzymes with the same function. The analysis of isozymes of a given enzyme system is usually done through gel electrophoresis. In this technique, plant tissues are ground in an extraction buffer and loaded into a gel. The gel usually consists of starch or polyacrylamide and any of a wide variety of buffers ranging in pH from 4.6 to 9.0 are used in electrophoresis (Shaw and Prasad, 1970). The enzymes migrate in the gel during electrophoresis according to their molecular weight, conformation, and net charge. After separation, zones of enzyme activity are resolved by incubating the gel in a specific activity stain. The banding pattern of an enzyme system is referred to as a zymogram. Bands produced by the same locus are referred to as allozymes and those produced by different loci are termed isozymes.

Isozymes have the following useful properties in plant breeding and genetics (Tanksley and Rick, 1980): (1) they are codominantly expressed, and both alleles in a diploid can be observed; (2) electrophoretic mobility of isozymes is not affected by environment; (3) sampling of tissue is not destructive to the plant; (4) isozyme genotypes can be deter-

mined on young seedlings in a matter of hours; and (5) isozymes have neutral effects on the overall phenotype of the plant. In genetic studies, isozymes have been used to differentiate plant genotypes (Weeden and Lamb, 1985), to separate accidental self-pollinations from hybrids in a breeding program (Soost et al., 1980), to determine phylogenetic relationships between species (Crawford, 1983), to analyze the genetics of a population (Brown and Weir, 1983), to identify loci controlling quantitative traits (Tanksley et al., 1982), or as selectable genetic markers for important horticultural traits (Rick and Fobes, 1974; Tanksley et al., 1984; Weeden et al., 1984).

Isozymes of tomato have been studied extensively. Using the polymorphism existing among several tomato wild species, a linkage map of isozyme loci has been constructed. At this time, 41 isozyme loci have been mapped, with at least one locus on each of the 12 tomato chromosomes (Bournival et al., 1988; Chetelat, 1989). For a list of enzyme abbreviations, see page v. A wild species, L. pennellii, is used commonly in tomato genetics, because of its great genetic distance from L. esculentum. Twenty-four of the 41 mapped isozyme loci are polymorphic between L. esculentum and L. pennellii. They are Prx-1, Skdh-1, Bnaq-1, Nir-1 and Dia-2 on chromosome 1; Est-7, Prx-2 and Fdh-1 on chromosome 2; Prx-7 on chromosome 3; Tpi-1, Pgm-2 and Adh-1 on chromosome 4; Dia-1 on chromosome 5; Aps-1 on chromosome 6; Got-2 on chromosome 7; Aps-2 on chromosome 8;

Est-2 on chromosome 9; Est-8 and Prx-4 on chromosome 10; Sod-1 on chromosome 11; Est-4, 6Pgdh-2, Pgi-1 and Aco-1 on chromosome 12.

Tomato isozyme loci have been used to map several genes of economic importance. Rick and Fobes (1974) reported tight linkage between Mi, a nematode resistance gene, and Aps-1 on chromosome 6. Tanksley et al. (1984) found tight linkage between a nuclear male-sterile locus, ms-10, and Prx-2 on chromosome 2. In breeding programs, Prx-2 and Aps-1 have been used extensively as selectable markers for ms-10 and Mi, respectively (Rick and Fobes, 1974; Medina-Filho and Stevens, 1980; Tanksley et al., 1984). Tanksley and Loaiza-Figueroa (1985) mapped the Lycopersicon gametophytic self-incompatibility locus, S, to chromosome 1 linked to Prx-1. Bournival et al. (1989, 1990) reported that the resistance in L. pennellii to race 1, 2, and 3 of Fusarium wilt were tightly linked to Got-2.

Quantitative traits of tomato have also been examined using isozymes. Tanksley et al. (1981) analyzed four quantitative traits (leaf shape, stigma exertion, fruit weight, and seed weight) and 12 polymorphic isozyme loci in a backcross population. They detected a significant correlation between mean heterozygosity (the portion of heterozygous isozyme loci in each backcross individual) and measurements for each of the quantitative traits. They concluded that the isozyme genotypes could be used to predict the quantitative

trait phenotypes. Furthermore, 21 loci controlling these traits were mapped using the 12 isozyme loci as markers (Tanksley et al., 1982).

Hypersensitive Resistance

Hypersensitivity appears to be a general resistance mechanism in plants to bacterial pathogens (Klement, 1982). A hypersensitive reaction (HR) occurs in plant species which normally are not susceptible to the bacterial species. Hypersensitivity in plants is commonly observed after inoculation with high concentrations of phytopathogenic pseudomonads and xanthomonads. Nonhost plant cell metabolism is severely disrupted and cell death soon follows. HR has been useful both as an aid for identification of bacteria and determination of host ranges (Klement, 1982). In all examples studied, HR was associated with vastly fewer bacteria per unit area of leaf than occurred in compatible, susceptible reaction (Klement et al., 1964; Lyon and Wood, 1976; Roebuck et al., 1978).

The HR is inducible (Meadows and Stall, 1981; Klement, 1982). Cell to cell contact between host and bacterial cells is required for induction of HR but not of the susceptible reaction (Lyon and Wood, 1976; Stall and Cook, 1979). A specific recognition event is presumed to occur during this phase (Keen and Holliday, 1982), but its nature is unknown (Staskawicz et al.,1984). The recognition is under genetic

control (Stall, 1987; Staskawicz et al., 1984) according to the model of a locus in the pathogen interacting with a complementary locus in the host (Flor, 1956). Length of the induction period is usually measured in plant tissues inoculated with high concentrations of bacteria. In such tests, induction time varies between approximately 1 and 5 hours after inoculation after which HR usually proceeds irreversibly (Klement, 1982).

Once HR is induced, host cells collapse following a latent period of variable length, during which biochemical events leading to cell collapse are presumed to occur (Klement, 1982). The cell collapse phase of HR occurs with an irreversible breakdown of membranes and plastids, and the mixing of cytoplasmic and vacuolar contents (Stall and Cook, 1966; Roebuck et al., 1978; Al-Mousawi et al., 1982). Inoculated leaf tissues lose turgor as metabolism is disrupted, and loss of electrolyte also occurs from tissues in the susceptible reaction, but the rate of increase of electrolyte leakage is slow and gradual in comparison with HR-induced losses. Concomitantly, bacteria cease multiplying (Klement et al., 1964), and their numbers may progressively decline over time (Cook and Guevara, 1984). Host cells may accumulate large amounts of antibiotics, possibly phytoalexins, prior to tissue collapse (Long et al., 1985). Release of these and acid vacuolar contents inhibit further bacterial multiplication (Klement et al., 1964; Long et al., 1985), and bacteria may die.

Hypersensitivity appears to be under genetic control. The inheritance of genes that are responsible for HR could be investigated in progenies that segregate for this trait. Bacterial spot of pepper caused by Xanthomonas campestris pv. vesicatoria (Xcv) is one of the best studied example. Three dominant genes for HR to Xcv have been described in pepper. The resistance gene, Bs1, was found in a heterogenous PI line of Capsicum annuum L. (Cook and Stall, 1963), the Bs2 gene in a PI line of C. chacoense L. (Cook and Guevara, 1984), and the Bs3 gene in C. annuum (Kim and Hartmann, 1985). Allelism tests have indicated that these three resistant genes are independent and dominant (Hibberd et al., 1987b). Near-isogeneic lines of the pepper cultivar Early Calwonder (ECW), differing only in the presence of Bs1, Bs2, or Bs3 have been developed. These have been used extensively in the classification of strains of Xcv and other basic research of HR. The phenotypic response and induction time of HR induced by these three resistance genes are different (Minsavage et al., 1990).

Inheritance of hypersensitive resistance in some other plant bacterial diseases has been studied and most of them appear to be controlled by single dominant genes in the host. For example, the HR in soybean to the bacterial blight organism (Pseudomonas syringae pv. glycinea) has been intensively studied. Four single dominant and nonallelic resistant

genes (Rpg1, Rpg2, Rpg3, and Rpg4) controlling HR have been reported in soybean (Mukherjee et al., 1966; Keen and Buzzell, 1991). Patel (1982) reported two race-specific dominant genes (Bp-1 and Bp-2) in cowpea for HR to bacterial pustule caused by Xanthomonas campestris pv. vignaeunguiculatae. Cotton genes for HR to bacterial blight caused by X. campestris pv. malvacearum are also dominant (Brinkerhoff et al., 1984). In tomato, hypersensitive resistance to P. syringae pv. tomato in accessions PI112215 of L. pimpinellifolium, PI129157 of L. hirsutum f. glabratum, and L. esculentum 'Ontario 7710' was controlled by a single dominant gene common to each of the three lines (Lawson and Summers, 1984b). However, Dessert, et al. (1982) reported that the hypersensitive resistance of cucumber to angular leafspot caused by Pseudomonas lachrymans was controlled by a single recessive gene.

Hypersensitivity is a widely occurring active defense system that occurs in most higher plants in response to all major groups of plant pathogens--viruses, bacteria, nematodes, and fungi. HR is widely considered as a mechanism of vertical resistance in plant fungal diseases (Vanderplank, 1982). Based on the description of Vanderplank (1982), vertical resistance is usually a race specific, monogenic or oligogenic trait. The mode of gene action of this type of resistance can be dominant, incomplete dominant, or recessive. In plant viral diseases, hypersensitivity is termed virus localization, because necrosis does not always appear. Many examples of

resistance to viruses operate by preventing spread of the virus from the site of initial infection. They are controlled by dominant, or less frequently by dosage-dependent genes and rarely controlled by recessive genes (Fraser, 1985).

Some disease resistance genes are sensitive to temperature. Generally, resistance fails at higher temperatures; such as, Sr6 for resistance in wheat to Puccinia graminis f. sp. tritici (Mayama et al., 1975). However, in the same pathosystem, examples of the converse are also known (Gousseau, et al., 1985). Both the expression and dominance relationships of alleles for resistance can be conditioned by temperature. A resistance gene can segregate as a completely dominant or completely recessive character under different temperatures, such as the temperature sensitive resistance allele Sr6 in wheat (Mayama et al., 1975).

Gene-for-Gene Relationship

The gene-for-gene hypothesis was first developed by Flor in 1935, in order to explain the infection results in the flax and flax rust system. The hypothesis stated that the genes controlling resistance in the host and avirulence in the pathogen are complementary (Flor, 1956). The genes for resistance or avirulence could be dominant (in most cases), recessive, or incompletely dominant. Studies of agricultural host-parasite systems have shown that such interactions do

occur quite commonly, but it has been demonstrated only in vertical resistance which is monogenic or oligogenic (Crute, 1985).

Following the development of recombinant DNA techniques, an avirulence gene was first cloned from P. syringae pv. glycinea by Staskawicz et al. (1984). Since then, many other avirulence genes have been cloned from pathogenic bacteria (Keen, 1990), but none from pathogenic fungi and virus. However, it has been demonstrated that the coat protein gene of TMV functions as an avirulence gene in the interaction with the N' resistance gene in tobacco (Knorr and Dawson, 1988). After the cloning of avirulence genes, it has been possible to prove the gene-for-gene interaction. The best example may be the Xcv - pepper system, because this is the only system in which near-isogenic lines containing different resistance genes have been used to demonstrate this relationship. Six avirulence genes have been cloned from Xcv (Whalen et al., 1988; Minsavage et al., 1990; Canteros et al., 1991). They do not only control the specificity at the cultivars level, but also at the host range level. The HR of each combination of resistance and avirulence genes displayed a unique phenotype. When Xcv strains carrying avrBs1 were infiltrated on ECW-10R (near-isogenic line of ECW containing Bs1 resistance gene), a dark, papery brown, confluent necrosis appeared within 8 to 12 hours. On ECW-20R containing Bs2, Xcv strains carrying avrBs2 induced a medium brown necrosis, which appeared within 12 to

36 hours. The phenotype of HR associated with interaction between Xcv strains carrying avrBs3 and ECW-30R containing Bs3 gene was a medium brown, confluent necrosis that appeared within 24 to 36 hours. The phenotype of the HR of pepper lines ECW and ECW-30R associated with avrBsT was a light brown, non-confluent necrosis, which appeared within 24 to 36 hours. On tomato, Xcv strains containing avrBsP induced a medium brown, confluent necrosis that appeared within 24 to 36 hours. On resistant tomato line Hawaii 7998, Xcv strains containing avrRxv induced a HR similar to the tomato-avrBsP combination.

The interactions between plant disease resistance genes and pathogen avirulence genes that initiate the plant HR have been thoroughly documented genetically (Flor, 1956). However, little is known about biochemical mechanisms that confer the genetic complementarity, because the functions of avirulence genes in the pathogen have generally not been deduced and the cloning of plant resistance genes has been difficult, since the gene products have not been identified.

CHAPTER 3
CHARACTERISTICS AND INHERITANCE OF THE HYPERSENSITIVITY INDUCED BY RACE 1 OF THE TOMATO GROUP OF XCV IN RESISTANT TOMATO LINE - HAWAII 7998

Introduction

A tomato accession of Lycopersicon esculentum Mill., Hawaii 7998 (H7998), is the only source with a high level resistance to bacterial spot caused by Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv) (Scott and Jones, 1986). This resistance is associated with a hypersensitive reaction (HR) (Jones and Scott, 1986). Hypersensitive resistance in plants to other bacterial diseases has been demonstrated to follow the "gene-for-gene" hypothesis (Keen, 1990). According to this hypothesis, the incompatible interaction between products of an avirulence gene in the pathogen and a complementary resistance gene in the plant results in the HR (Flor, 1956). The avirulence and resistance genes usually are dominant (Crute, 1985; Keen, 1990). An avirulence gene, avrRxv, has been cloned from DNA of Xcv (Whalen et al., 1988). An HR in plants of H7998 is caused by strains of Xcv which contain avrRxv and not by strains that lack it (Whalen, unpublished data). However, the inheritance of bacterial spot resistance in H7998 is quantitative under field conditions (Scott and Jones, 1986). Since this type of inheritance is in

variance with the gene-for-gene hypothesis, more detailed studies of the inheritance of the HR in H7998 is needed to confirm the quantitative inheritance of the resistance.

Under greenhouse conditions, the resistant lines cannot be distinguished easily from susceptible tomato lines after inoculation. Staskawicz (personal communication to Dr. R. E. Stall) noted that at relatively low temperatures (24°C) the difference between H7998 and a susceptible line became more distinct.

The objectives of this chapter were: (1) to study the effect of temperature on HR by comparing the patterns of bacterial growth curves and changes in electrolyte leakage; (2) to determine the HR phenotype of plants in the F_1 and F_2 populations of a cross between H7998 and susceptible lines of L. esculentum using the optimum conditions for HR development; and (3) to compare the HR phenotype of plants in the F_2 population inoculated with a wild strain and a wild strain containing the avirulence gene, avrRxv.

Materials and Methods

Plant materials

H7998 were used as the resistant parents, and plants from the breeding line Fla.7060 (7060) were used as the susceptible parents. These two genotypes were used to generate F_1 and F_2 generations. In some tests, plants of cultivar Bonny Best

(BB) were used as susceptible controls. All plants were grown in a greenhouse and transferred to a growth chamber for testing. Optimum conditions for vigorous growth were maintained. For determination of internal bacterial populations and electrolyte leakage, plants were topped above the fully expanded sixth true leaf. For other tests, plants were topped above the fully expanded fourth true leaf. Approximately 7 days after topping, plants were used for inoculations.

Bacterial cultures and strains

Strains of Xcv were stored in sterile tap-water and were subcultured on nutrient agar. Transfers were made to nutrient broth to obtain suspensions for inoculation. After incubation and shaking overnight at 28°C, bacterial suspensions were prepared by centrifugation, followed by resuspension of the bacterial pellet in sterile tap-water, and standardization of suspensions to an absorbance of 0.3 at a wavelength of 600 nm (concentration about 5×10^8 cfu/ml). Bacterial suspensions with lower concentrations were made from the above suspension.

Strains 75-3 or 90-14 were used in the inoculations. These strains belong to race 1 of the tomato group of Xcv (XcvT) which induce HR in plants of H7998 but not in BB or 7060. A strain from Brazil, XV56, obtained from Dr. J. B. Jones, Gulf Coast Research and Education Center, Bradenton, FL, was used to induce a susceptible reaction in H7998, BB,

and 7060. XV56 was used as the recipient for pXV9100, a plasmid maintained in E. coli DH5 alpha, which carries the avirulence gene, avrRxv, from 75-3. The plasmid was obtained from Dr. M. C. Whalen, Colby College, Waterville, Maine.

Inoculation

In tests to determine internal populations in inoculated leaves, approximately 2 cm^2 of leaf area were infiltrated with a bacterial suspension at a concentration of 5×10^5 cfu/ml. For measuring electrolyte leakage, inoculum concentrations of 5×10^8 cfu/ml were infiltrated into approximately 5 cm^2 of leaf area. For observations of the HR phenotype in the progenies, the high concentration of inoculum (5×10^8 cfu/ml) was infiltrated into about 1 cm^2 of leaf area. Infiltrations were accomplished by forcing suspensions into the intercellular spaces with a hyperdermic syringe and needle.

Effect of temperature on electrolyte leakage and growth curve

Plants of H7998 and BB were inoculated with strain 75-3. In one set of plants, the internal bacterial growth was determined every 24 hours after inoculation. In another set the change in electrolyte leakage was determined every 12 hours after inoculation. With each set of plants, onehalf were incubated in a growth chamber at 24°C and 60% humidity with a 16-hr light period and the other half at 30°C under the same conditions. For the growth curve experiments, leaf

samples consisting of 1 cm^2 were triturated in 1 ml of sterile tap-water. Bacterial numbers were determined by a standard dilution-plate method. For measuring electrolyte leakage, leaf samples consisting of 3 cm^2 were individually added to 3 ml of deionized water. Conductivity of the water was measured by the procedure described by Hibberd et al. (1987a). In all tests each treatment was replicated three times.

Growth curve of Xcv in F_1 plants

The intensity of HR of F_1 plants was associated with the growth of strain 75-3 in the leaves. Plants of H7998 and 7060 were used as controls. Bacterial populations were determined every 24 hours after inoculation and by the methods described in the above section. Three leaflets were used as replications. The tests were performed at 24°C in growth chambers with a 16-hr light period.

Development of necrosis in an F_2 population

A population of 100 F_2 plants of the cross between H7998 and breeding line 7060 was used in this test. All plants were infiltrated with a suspension of 75-3 containing 5 x 10^8 cfu/ml. The percentage of the total infiltrated area that was necrotic was rated every 4 or 8 hours after infiltration. The following rating scale was used: '0' as no necrosis; '1' as 1-20% necrotic area; '2' as 21-40% necrotic area; '3' as 41-60% necrotic area; '4' as 61-80% necrotic area; and '5' as

confluent necrosis. Plants of H7998 and 7060 were used as control. The tests were performed at 24°C in the growth chambers with a 16-hr light period.

Development of necrosis of plants of the F_2 population inoculated with a virulent strain containing avrRxv

A population of 129 F_2 plants was used in this test. Bacterial suspensions of strain 90-14 and XV56 containing pXV9100, each containing 5 x 10^8 cfu/ml, were infiltrated into leaflets on the opposite sides of the same leaf. The percentage of infiltrated area that was necrotic was rated every 8 hours after infiltration. The rating scale was the same as the above test. Plants of H7998, F_1, and 7060 were used as controls. The plants were kept in a growth room at 24°C and 60% humidity with a 12-hr light period.

Results

Effect of temperature on electrolyte leakage and growth curve

The growth curves of XcvT strain 75-3 in plants of H7998 and BB were similar at 30°C (Fig. 3.1). The bacterial populations started at approximately 10^4 cfu/cm^2 and increased logarithmically during the first 3 days. The maximum (approximately 10^8 cfu/cm^2) was reached 6 days after infiltration. At the end of the test, yellowing and slightly water-soaked symptoms were observed in plants of H7998 and BB at 30°C. However, there was a clear difference between the

growth curves from the plant types at 24°C (Fig. 3.1). In plants of H7998, the population started as 7.3×10^3 cfu/cm^2 and the logarithmic growth continued for 2 days. The population became stable afterward, about 10^6 cfu/cm^2. At this moment, slight necrosis was noted. In plants of BB, the population started as 3.9×10^3 cfu/cm^2 and reached the maximum (7.1×10^7 cfu/cm^2) 3 days after infiltration. No symptoms were observed in the plants of BB until the end of the test at 24 C.

The patterns of electrolyte leakage were similar in H7998 at 24° and 30°C (Fig. 3.2). The conductivity of water containing tissues from the infiltrated areas increased after inoculation and reached the maximum at 24 hours. In the plants of BB, conductivity of water-containing inoculated tissues did not increase during the first 12 hours at either temperature. However, conductivity was higher from tissues of plants incubated at 30°C afterward.

Growth curve of Xcv in F_1 plants

F_1 plants of the cross between H7998 and 7060 displayed an intermediate phenotype based on growth of strain 75-3 in leaves of the three plant types (Fig. 3.3). The bacterial population in plants of H7998, the F_1, and 7060 started at about the 10^4 cfu/cm^2 level and differences appeared 2 days after infiltration. Then slight necrosis appeared in the plants of H7998, but no symptoms occurred in the F_1 and 7060

plants. Four days after infiltration, the populations in plants of H7998, F_1, and 7060 were 1.9 x 10^6, 1.3 x 10^7, and 8.9 x 10^7 cfu/cm^2, respectively.

Inheritance of the hypersensitive resistance in H7998

Confluent necrosis occurred from 36 to 60 hours in H7998 plants and from 84 to 96 hours in 7060. The time for appearance of confluent necrosis was different among the plants of the F_2 population. Continuous variation occurred among the plants with regard to ratings of necrosis and no distinct groups were observed at any time (Table 3.1).

Development of necrosis of plants of the F_2 population inoculated with a virulent strain containing avrRxv

Confluent necrosis was observed in plants of H7998 from 24 to 40 hours after infiltration of strain 90-14, and from 32 to 48 hours after infiltration of strain XV56 containing pXV9100. Most plants of H7998 had similar reactions to both strains as indicated by the average ratings of necrosis (Table 3.2 and 3.3). However, strain 90-14 caused a faster HR development than strain XV56 containing the cloned avirulence gene in one of the seven tested plants. Slight variation of HR development occurred among H7998 plants inoculated with each strain.

During the first 64-hour period, the average necrosis ratings were slightly higher in the F_1 plants infiltrated with

strain 90-14 than with strain XV56 containing avrRxv. In the ensuing time period, the average of ratings was similar with both strains (Table 3.2 and 3.3). Seven of the 15 tested F_1 plants showed similar reactions to both strains during the first 64-hour period. The rest of the F_1 plants had faster development of necrosis in leaflets infiltrated with strain 90-14.

All seven tested plants of breeding line 7060 had faster development of necrosis in the leaflets infiltrated with strain XV56 containing the avirulence gene avrRxv than that in the leaflets infiltrated with 90-14. Thus, the average ratings of necrosis for the susceptible plants were higher in the leaflets infiltrated with strain XV56 containing the clone at all time periods (Table 3.2 and 3.3).

Continuous variation in development of necrosis was observed in the F_2 population, no matter which strain was used (Table 3.2 and 3.3). In order to determine the similarity of reactions to strain 90-14 and XV56 with the avirulence gene in the F_2 population, correlation coefficients were calculated between the ratings of both strains at each time of rating. They were 0.78, 0.79, 0.63, 0.63, 0.70, 0.67, 0.63, 0.54, 0.60, 0.58, 0.53, 0.47, and 0.33. The significance levels for all the above correlation coefficients were $p=0.0001$.

The percentages of plants in the F_2 population that increased in the level of necrosis in the infiltrated area during consecutive rating periods were calculated and the

changes over time are shown in Fig. 3.4. When strain 90-14 was used, the percentage of plants which increased in necrosis between rating periods increased right after infiltration but the first peak was reached during the 24-32 and 32-40 hour periods. Afterward, the percentage of plants that with increased necrosis decreased rapidly and reached the lowest point during the 48-56 hour period. Other peaks of increase in necrosis development were observed during the 64-72 and 88-96 hour periods. When strain XV56 with the avirulence gene was used as inoculum, the percentage of the plants which increased in necrosis increased gradually after infiltration, and the first peak was reached during the 40-48 hour periods. The largest peak occurred right after the first peak, and the highest point was reached during the 64-72 hour period.

Discussion

The hypersensitive reaction in H7998 induced by race 1 of XcvT was shown to be temperature sensitive. The interaction appeared to change from incompatible to compatible based on bacterial growth when the incubation temperature was 24°C instead of 30°C. This change was not indicated by electrolyte leakage, since H7998 had similar patterns of change under both temperatures. The high inoculum concentration used in the electrolyte leakage test in contrast to the low inoculum level used in the growth tests might overcome the effect of

temperature. However, the HR expressed in plants of H7998 was more easily observed at 24°C, because the differential with the susceptible reaction was greater. The general case is for incompatible interactions to fail at higher temperatures, such as the hypersensitive resistance in cv. Harosoy soybeans to race 1 of Pseudomonas syringae pv. syringae (Keen et al., 1981).

The HR in H7998 to race 1 of XcvT was inherited as a quantitative trait. This was evident by the intermediate HR phenotype of the F_1 plants and the continuous variation of necrosis reaction in the F_2 population. The quantitative inheritance of the HR is unique in plant-microbe interactions. It is possible that more than one resistance genes in H7998 interacts with strains of race 1 of XcvT and some of the resistance genes might be in a heterozygous condition. If this were true, there should be several corresponding avirulence genes in the causal bacterium, based on the gene-for-gene hypothesis which has been demonstrated in the Xcv - pepper system (Minsavage et al., 1990). That this might occur was supported by the evidence that (1) some of the plants of H7998 and F_1 had different rates of HR development to the wild type of race 1 of XcvT (90-14) and XV56 containing avrRxv; and (2) in the F_2 population, the first peak was larger and observed earlier in the dynamics of development of necrosis when the wild type was used as inoculum. Since the resistance corresponding to the avirulence gene, avrRxv, was also a

quantitative trait, the mode of inheritance of possible other resistance genes cannot be determined at this moment.

It has been determined that the genetic background was quite different between XV56 and the XcvT strains (Stall, unpublished data). Strain XV56 might be more aggressive than strain 90-14, because the necrosis occurred earlier in the leaflets of the susceptible breeding line 7060 infiltrated with strain XV56 containing pXV9100. This could account for the gradual decrease in correlation coefficients in comparisons of ratings of necrosis caused by the wild strain and the XV56 containing pXV9100.

Table 3.1 Frequency distribution of plants of the F_2 population according to ratings for necrosis after infiltration with strain 75-3.

Hours[a]	5	4	3	2	1	0[c]	H7998	7060
24	0[b]	0	4	8	39	47	2.9[d](2-3)[e]	0.0d
30	0	3	2	12	39	42	3.4 (2-4)	0.1 (0-1)
36	2	2	7	17	46	24	4.3 (3-5)	0.1 (0-1)
40	2	3	12	18	47	16	4.4 (3-5)	0.3 (0-1)
48	3	8	12	24	44	7	4.6 (3-5)	0.5 (0-1)
54	7	15	23	25	25	3	4.7 (4-5)	1.0 (0-2)
60	24	17	24	18	14	1	5.0	1.4 (0-3)
64	38	17	19	14	9	1	5.0	1.9 (0-4)
72	49	19	12	14	4	0	5.0	2.4 (1-4)
78	65	8	15	9	1	0	5.0	3.4 (2-4)
84	74	12	8	4	0	0	5.0	4.4 (4-5)
88	79	13	6	0	0	0	5.0	4.5 (4-5)
96	89	9	0	0	0	0	5.0	5.0

[a] Hours after infiltration.
[b] Number of plants out of 94 that had indicated rating.
[c] Rating scale for necrosis were: '0'- no symptom; '1'- less or equal to 20% of the infiltrated area was necrosis; '2'- 40% necrosis; '3'- 60% necrosis; '4'- 80% necrosis; and '5'- confluent necrosis.
[d] Average necrosis rating of seven plants.
[e] Range of ratings of the average.

Table 3.2 Frequency distribution of plants of the F_2 population according to ratings for necrosis after infiltration with strain 90-14.

Hours[a]	5	4	3	2	1	0[c]	H7998	F_1	7060
16	0[b]	0	0	1	4	124	0.7[d] (0-3)[g]	0.0[e]	0.0[f]
24	0	0	1	1	15	112	2.4 (0-3)	0.0	0.0
32	0	1	5	10	41	72	4.4 (3-5)	0.4 (0-1)	0.0
40	3	7	14	12	42	51	5.0	0.9 (0-2)	0.0
48	9	6	12	18	40	44	5.0	1.1 (0-3)	0.0
56	10	11	9	22	39	38	5.0	1.2 (0-3)	0.0
64	14	9	15	26	39	26	5.0	1.6 (1-3)	0.1 (0-1)
72	24	18	22	24	32	9	5.0	2.3 (1-4)	0.4 (0-1)
80	38	21	24	24	19	3	5.0	3.0 (1-5)	1.3 (0-3)
88	50	22	27	16	12	2	5.0	3.6 (2-5)	2.0 (0-4)
96	70	24	18	14	3	0	5.0	4.2 (3-5)	2.8 (1-5)
104	96	21	9	2	1	0	5.0	4.7 (4-5)	4.0 (3-5)
112	112	12	3	1	1	0	5.0	5.0	4.8 (4-5)

[a] Hours after infiltration.
[b] Number of plants out of 129 that had indicated rating.
[c] Rating scale for necrosis were: '0'- no symptom; '1'- less or equal to 20% of the infiltrated area was necrosis; '2'- 40% necrosis; '3'- 60% necrosis; '4'- 80% necrosis; and '5'- confluent necrosis.
[d] Average necrosis rating of 9 plants.
[e] Average necrosis rating of 15 plants.
[f] Average necrosis rating of 9 plants.
[g] Range of ratings of the average.

Table 3.3 Frequency distribution of plants of the F_2 population according to ratings for necrosis after infiltration with strain XV56 containing pXV9100

Hours[a] 5	4	3	2	1	0[c]	H7998	F_1	7060
16 0[b]	0	0	0	5	124	0.4[d] (0-1)[g]	0.0[e]	0.0[f]
24 0	0	1	1	7	120	2.0 (1-3)	0.0	0.0
32 0	1	0	1	19	108	4.1 (3-5)	0.1 (0-1)	0.0
40 1	1	2	6	18	101	4.8 (4-5)	0.2 (0-1)	0.0
48 4	4	2	6	37	76	5.0	0.4 (0-1)	0.0
56 8	1	3	10	37	70	5.0	0.6 (0-2)	0.1 (0-1)
64 10	3	3	12	41	60	5.0	0.8 (0-2)	0.3 (0-1)
72 21	17	17	24	33	17	5.0	2.0 (1-4)	2.2 (1-3)
80 43	18	16	16	24	12	5.0	2.8 (1-5)	3.3 (1-5)
88 62	13	13	16	21	4	5.0	3.5 (1-5)	4.2 (3-5)
96 77	14	12	14	11	1	5.0	4.0 (1-5)	4.7 (4-5)
104 93	15	9	7	5	0	5.0	4.4 (1-5)	5.0
112 107	10	7	3	2	0	5.0	4.8 (2-5)	5.0

[a] Hours after infiltration.
[b] Number of plants out of 129 that had indicated rating.
[c] Rating scale for necrosis were: '0'- no symptom; '1'- less or equal to 20% of the infiltrated area was necrosis; '2'- 40% necrosis; '3'- 60% necrosis; '4'- 80% necrosis; and '5'- confluent necrosis.
[d] Average necrosis rating of 9 plants.
[e] Average necrosis rating of 15 plants.
[f] Average necrosis rating of 9 plants.
[g] Range of ratings of the average.

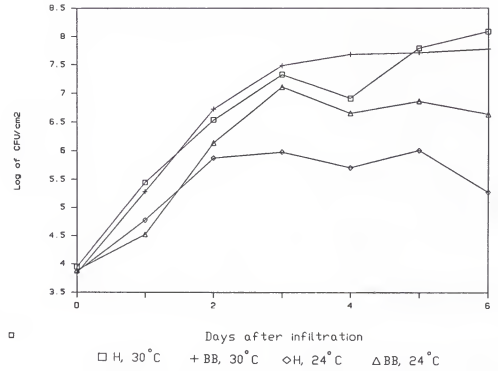

Figure 3.1 Internal bacterial population in plants of Hawaii 7998 (H) and Bonny Best (BB) infiltrated with strain 75-3 at 24 C and 30 C.

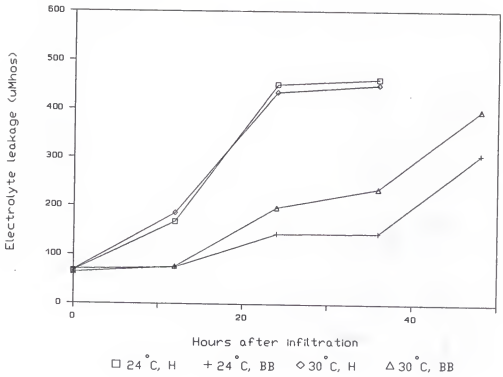

Figure 3.2 Changes in electrolyte leakage in plants of Hawaii 7998 (H) and Bonny Best (BB) infiltrated with strain 75-3 at 24 C and 30 C.

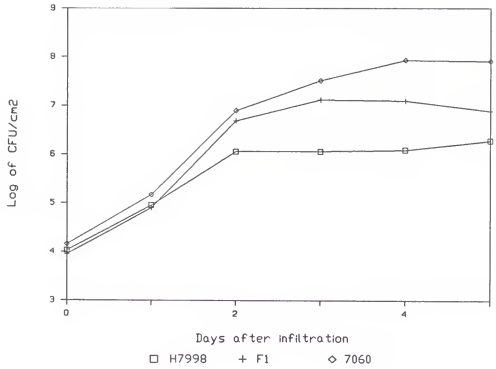

Figure 3.3 Internal bacterial population in plants of Hawaii 7998 (H7998), F_1, and breeding line 7060 infiltrated with strain 75-3 and incubated at 24 C.

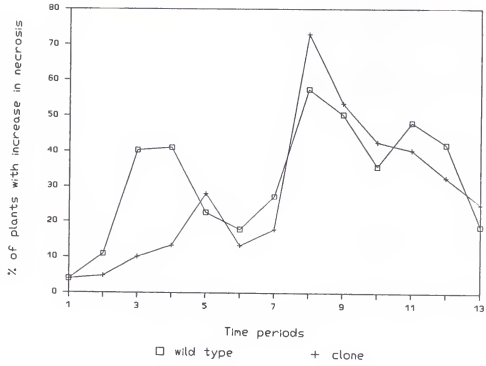

Figure 3.4 Dynamics of development of necrosis in the F_2 population infiltrated with strain 90-14 (wild type) and XV56::PXV9100 (clone). Time period 1 (0-16 hours), 2 (16-24 hours), 3 (24-32 hours), 4 (32-40 hours), 5 (40-48 hours), 6 (48-56 hours), 7 (56-64 hours), 8 (64-72 hours), 9 (72-80 hours), 10 (80-88 hours), 11 (88-96 hours), 12 (96-104 hours), 13 (104-112 hours).

CHAPTER 4
MAPPING OF THE HYPERSENSITIVITY ASSOCIATED GENES IN THE RESISTANT TOMATO LINE - HAWAII 7998.

Introduction

Bacterial spot caused by Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv) is one of the most important diseases of tomatoes in regions where high temperatures and heavy rainfall occur during the cropping season. The high incidence of bacterial spot probably occurs because chemical control of the disease is inefficient and resistant cultivars of tomato have not been developed to date (Pohronezny et al., 1986). However, a single accession of Lycopersicon esculentum Mill, Hawaii 7998 (H7998) has been identified as a source of resistance which is being transferred to commercial lines (Scott and Jones, 1986). This resistance is associated with a hypersensitive reaction (HR) which can be recognized after infiltration of high inoculum levels into leaves (Jones and Scott, 1986).

Hypersensitive resistance in plants to some other bacterial diseases has been demonstrated to follow the "gene-for-gene" hypothesis (Keen, 1990; Minsavage et al., 1990). According to this hypothesis, the incompatible interaction between an avirulence gene in the pathogen and a corresponding resistance gene in the plant results in the HR (Flor, 1956).

An avirulence gene, avrRxv, has been cloned from DNA of Xcv (Whalen et al., 1988). An HR in plants of H7998 is caused by strains of Xcv that contain avrRxv and not by those that lack it (Stall, unpublished data). However, resistance in H7998 seems to be more complex. First, F_1 plants between H7998 and a susceptible tomato displays an intermediate HR intensity; and second, continuous variation of HR intensity occurs among the F_2 progeny of this cross (Wang et al., 1991). Thus, this HR-associated resistance appears to be a quantitative trait in tomato. Scott and Jones (1989) reported that the inheritance of the H7998 resistance to bacterial spot conformed to a polygenic system in field tests.

To further pinpoint the genetic determination of resistance to bacterial spot in H7998, an association of hypersensitivity with molecular markers would be beneficial. Molecular markers in tomato have been used to study the inheritance and linkage relationships of monogenic disease resistance loci. Examples of these include the resistance to root knot nematode, Mi, linked to Aps-1 (Rick and Fobes, 1974), the resistance to race 3 of Fusarium oxysporium f.sp. lycopersici, I-3, linked to Got-2 (Bournival et al., 1989), and the resistance to tobacco mosaic virus, Tm-2a, linked to specific RFLP markers (Young et al., 1988). Molecular markers also have been used to study the inheritance of quantitative traits in tomato. For example, twenty-one loci controlling four quantitative traits (leaf shape, stigma

exertion, fruit weight, and seed weight) were mapped using 12 isozyme loci as markers (Tanksley et al., 1982). In this report, the use of isozyme markers to study the inheritance of the HR related resistance in H7998 is described. Due to the lack of polymorphism of isozyme loci within L. esculentum, interspecific progenies between H7998 and a susceptible wild tomato, L. pennellii, were used.

Materials and Methods

Plant materials

H7998 and the wild species L. pennellii accession LA 716 were used to produce plants of the following generations: an interspecific F_1 hybrid, F_2, backcross to the resistant parent (H7998 x [H7998 x LA 716], BCP1), and backcross to the susceptible parent ([H7998 x LA 716] x LA 716, BCP2). LA 716 was used as the susceptible parent to bacterial spot because a large number of its enzyme loci are polymorphic with respect to H7998. Unilateral compatibility between the two species required the use of L. esculentum as the pistillate parent. In some experiments, Bonny Best (BB), a susceptible cultivar of L. esculentum, was used as a control. In preliminary tests, Bonny Best was as susceptible as LA 716, which was determined by the patterns of growth curves and electrolyte leakage.

Isozyme analysis

The techniques of starch gel electrophoresis and enzyme staining used in this study have been described elsewhere (Rick et al., 1977; Tanksley and Rick, 1980; Vallejos, 1983; Bournival et al., 1988). The genotype of the following isozyme loci were determined in the F_2 plants : Prx-1, Skdh-1, and Bnag-1 on chromosome 1; Est-7, and Prx-2 on chromosome 2; Prx-7 on chromosome 3; Pgm-2 on chromosome 4; Prx-5 on chromosome 5; Aps-1 on chromosome 6; Got-2 on chromosome 7; Aps-2 on chromosome 8; Est-2 on chromosome 9; Prx-4 on chromosome 10; Sod-1 on chromosome 11; Est-4, 6Pgdh-2, and Pgi-1 on chromosome 12. One morphological locus Pn on chromosome 8 was also examined. The genotypes of the marker loci determined in BCP1 plants were the same as those in F_2 plants except Prx-2 and Prx-5 were not determined. The genotypes of the marker loci determined in BCP2 plants were the same as those in F_2 plants except Bnag-1, Prx-2, Prx-5, Pn, Est-2, Sod-1, and 6Pgdh-2 were not determined.

Inoculum source

Strain 90-14 of race 1 of the tomato group of Xcv (XcvT) strains was used in inoculations. Inoculum was prepared from cultures that had been grown in nutrient broth (DIFCO) with continuous shaking for 24 hr at 30°C. Cultures were centrifuged and the bacterial pellets were resuspended in sterilized tap-water. The bacterial suspension was standardized to about

5 x 10^8 cfu/ml (O.D. 600nm = 0.3). Inocula with different concentrations were obtained by dilution from the above suspension.

Rating of hypersensitive reaction

The BCP1, BCP2, and F_2 populations were tested individually. The parental types of each population were used as controls. All plants were grown in a greenhouse and topped above the fully expanded fourth-true leaf. Approximately 7 days after topping, plants were moved to a growth room at 24°C and 60% humidity with a 16-hr light period. Approximately 1 cm^2 of one leaflet of the fourth true leaf was infiltrated with a bacterial suspension by forcing the suspension into the intercellular spaces using a syringe fitted with a 26-gauge needle and the leaflet used for each plant was at the same position (Klement et al., 1964). The percentage of the total infiltrated area that was necrotic was estimated every 8 hours after infiltration. The following rating scale was used : '0' as no necrosis; '1' as 1-20% necrotic area; '2' as 21-40% necrotic area; '3' as 41-60% necrotic area; '4' as 61-80% necrotic area; and '5' as confluent necrosis. Plants with ratings greater than that of susceptible controls were considered to be hypersensitive to the bacterial pathogen.

The relationships between necrosis rating, electrolyte leakage and internal bacterial population

Three characteristics of HR caused by bacterial pathogen are commonly used for verifying that a particular plant produces HR to a bacterial pathogen: (1) rapid development of confluent necrosis; (2) rapid increase of electrolyte leakage; and (3) lower internal bacterial population compared to susceptible host plant (Klement, 1982). The necrosis ratings described previously were used to determine the rapidity of necrosis and were central for determinations of linkages with enzyme markers. However, there was concern about the accuracy of this rating system for HR. Thus, the relationships between the rating system, the electrolyte leakage, and internal bacterial population were examined.

Individual BCP1 plants with different speeds of development of necrosis were selected based on necrosis rating. Cuttings were made from each plant in the following tests. Cuttings of H7998 and Bonny Best were used as resistant and susceptible controls. For measuring electrolyte leakage, tested plants were infiltrated with bacterial suspension (5×10^8 cfu/ml). Electrolyte leakage was measured every 12 hours after infiltration according to the procedure previously described (Hibberd et al., 1987b). Another set of cuttings was used in the population test. Leaves were infiltrated with bacteria at a concentration of 5×10^5 cfu/ml. The bacterial population in the infiltrated area was

determined daily by dilution plate method after infiltration according to the procedure of Hibberd et al. (1987b). The relationships between necrosis rating, electrolyte leakage and internal population were determined by the rank order correlation coefficient which measures the monotonic association between variables, because linear association among these three variables does not exist (Ott, 1988).

Data analysis

Data sets for each experiment were handled using the 1-2-3 Access System (Lotus Development Corporation). All statistical analyses were performed on a PC computer using the Statistical Analysis System (SAS Institute, SAS Circle, Box 8000, Cary, North Carolina).

Results

Segregation of hypersensitive reaction

Confluent necrosis (rating of 5) was observed between 24 and 48 hours after infiltration in plants of H7998, and 3 to 5 days after infiltration in plants of L. pennellii (Table 4.1, 4.2, and 4.3). Intermediate development of necrosis was observed in the F_1 plants (Table 4.3). However, the level of necrosis in F_1 plants was much closer to that of the L. pennellii parent. The reactions of the parental controls were not identical, since the BCP1, BCP2, and F_2 populations were

tested at different times. Continuous variation in amount of necrosis at all time periods occurred among plants in the backcrosses and F_2 populations (Table 4.1, 4.2, and 4.3). This clearly indicated the quantitative nature of the inheritance of the HR from H7998.

The percentages of plants of each parental control with an increase in the necrosis rating at each time interval were calculated and plotted (Fig. 4.1). The necrosis developed in the plants of H7998 due to HR was occurred during 8 to 48 hours and the fastest development was observed at the 24-32 hours period (Fig. 4.1). The dynamic curve of the F_1 plants was similar to the curve of H7998, but the peak for the fastest necrosis development occurred later during 88-96 hours period. The development of necrosis in the F_1 plants was intermediate but closer to the susceptible plants. Necrosis development of the LA 716 plants occurred the latest. At the end of experiment, few of the plants of LA 716 did not show confluent necrosis symptoms.

The dynamics of development of necrosis were different among the BCP1, BCP2, and F_2 populations (Fig. 4.2). This was evident when the percentages of plants in the population that increased in the level of necrosis in the infiltrated area during consecutive rating periods were calculated and plotted (Fig. 4.2). In the BCP1 population, the percentage of the plants which increased in necrosis increased soon after infiltration but the highest point was reached during the 24-

32 and 32-40 hour periods. At this moment, all the plants of the resistant parent were rated '5' and necrosis in the F_1 plants was just visible (Fig. 4.1). Afterward, the percentage of BCP1 plants with increased necrosis decreased rapidly and reached the lowest point during the 48-64 hour period. Another moderate increase in necrosis development was observed during the 64-96 hour periods. During this time, the average rating of the F_1 plants was increased from 0.6 to 4.0.

Necrosis of the infiltrated areas in the plants of the BCP2 population developed slowly (Fig. 4.2). For the first 40 hours after infiltration, only 10.3% of the population increased in necrosis. Then the percentage of plants with increase in necrosis raised gradually and reached the highest point during the 73-80 hour period. During this period, the average rating of the F_1 plants increased from 1.1 to 2.3, and the average rating of the plants of L. pennellii increased from 1.0 to 1.4 (Table 4.2).

Intermediate change in development of necrosis was observed in the F_2 population (Fig. 4.2). The percentage of plants with necrosis increased soon after infiltration, became steady from 16 to 104 hours and increased afterward. In the F_2 test, all H7998 plants showed confluent necrosis at 48 hours after infiltration and the F_1 plants started to show necrosis at 72 hours and all of them had the rating of '5' at 104 hours. The plants of L. pennellii started to show necrosis at 80 hours and the average rating was 4.4 at 128 hours.

Relationships among HR rating, electrolyte leakage, internal bacterial population

Seven plants with different rates of necrosis after inoculation were selected from the BCP1 population. Plants numbered 50, 133, and 202 had similar development of HR to H7998 and were classified as resistant. Plant no. 103 was classified as susceptible which had the lowest necrosis development at all the time periods. Three plants numbered 70, 146, and 42 had intermediate development of HR and were selected as an intermediate group. Three days after infiltration, differences appeared in internal bacterial populations among the selected plants. Lower internal bacterial populations were associated with plants of the resistant group. Five days after infiltration, bacterial population in the resistant group was significantly less from the intermediate and susceptible group (Table 4.4). Differences in electrolyte leakage from inoculated tissues occurred 24 hours after infiltration. Electrolyte leakage was greatest from the leaves of the resistant group (Table 4.5). At 36 hours after infiltration, electrolyte leakage from leaves of the resistant group was significantly different from that of the intermediate and susceptible groups. Rank order correlation coefficients among HR rating, electrolyte leakage, and internal populations were calculated. Except for the correlations between 16 hr and 24 hr HR ratings with population, the correlations among these three variables were

significant ($p <= 0.05$ level). Time periods of 48 hr and 56 hr had the best correlation with both electrolyte leakage and internal population (Table 4.6). Thus, the rating system is sufficient to represent the HR development.

Isozyme analysis

Significant deviations from Mendelian ratios were detected at several loci in one or all progenies (Table 4.7). Most of the deviations were detected in the backcross where L. esculentum was the recurrent parent; eight out of 16 markers on 6 chromosomes were skewed significantly; they were Bnag-1 on chromosome 1, Est-7 on chromosome 2, Est-2 on chromosome 9, Prx-4 on chromosome 10, Sod-1 on chromosome 11, and Est-4, 6Pgdh-2, Pgi-1 on chromosome 12. In the F_2 population, only four out of 18 markers were skewed significantly. They were Prx-1 on chromosome 1, Aps-1 on chromosome 6, Sod-1 on chromosome 11, and Pgi-1 on chromosome 12. Deviations from the expected 1:1 ratio in a L. pennellii x L. esculentum backcross were reported previously (Rick, 1969; Bournival et al., 1989). However, this is the first report of significant skewing on chromosome 2 and 9. All other marker loci assorted as previously reported (Tanksley and Rick, 1980; Chetelat, 1989). Based on the BCP1 data the distance between linked markers were as follows: Prx-1 --30.4cM--Skdh-1--9.1cM--Bnag-1 on chromosome 1; Est-4--26.9cM--6Pgdh-2--13.9cM--Pgi-1 on chromosome 12. Previous reported distance was Prx-1--48cM--

[Skdh-1, Bnaq-1] and Est-4--8cM--6Pgdh-2--16cM--Pgi-1 (Chetelat 1989). Differences of map distance from previous reports were expected because recombination frequencies are known to depend on both the environment and the genotypes involved.

Linkages between isozyme markers and genes associated with the hypersensitive reaction using necrosis rating

Traditionally, the means of the studied phenotype of the genotypic classes at each segregating enzyme locus were statistically compared to test for possible linkages between enzyme loci and the studied trait. In this research, due to the limited range (from 0 to 5) of the rating scale, a one-tailed nonparametric analysis of variance - the Wilcoxon rank sum test was used to analyze the backcross data and an nonparametric unbalanced design analysis of variance was used for the F_2 data.

The plants of BCP1, BCP2 and F_2 populations displayed continuous variation of necrosis of infiltrated area at each time of rating. This implied that the genes controlling HR were segregating among the progenies and the effect of genes might accumulate over time. Thus, it is possible to detect the linkages by using the necrosis rating as criteria.

Significant differences between the means of different genotypes was detected for several enzyme loci (Table 4.8). These were Prx-1, Skdh-1, Got-2, Pn, Prx-4, and Sod-1 which

all had significant higher rating values in the homozygote group. Another linkage detected with Pgm-2 behaved the opposite way in which the heterozygous group had higher rating values. The linkages with Prx-1, Skdh-1 on chromosome 1, and with Pgm-2 on chromosome 4, were detected from 24 to 88 hours after infiltration (Table 4.8) which suggested that the linked genes have large effect on HR and are close to the marker loci. Other linkages detected with Got-2, Pn, Prx-4 and Sod-1 only appeared at one, or few time periods which suggest that these genes have small effect or they are far from the marker loci.

In the F_2 population, significant differences were detected with nine enzyme loci with the necrosis ratings at different time periods. They were Prx-1, Skdh-1, Bnag-1, Est-7, Prx-2, Pgm-2, Prx-5, Est-2, and Prx-4. However, the linkages with Prx-1, Skdh-1, Est-7, Prx-2, and Pgm-2, had higher significance level than the other linkages and most of them were detected during 32 to 104 hours after infiltration (Table 4.8). These suggested that the genes linked to these five markers might have larger effects on HR.

In the BCP2 population, significant difference was detected with five enzyme loci. They were Est-7, Pgm-2, Aps-2, Prx-4, and Pgi-1. The time period when these linkages were detected was from 48 to 112 hours. The effect of the genes linked to Aps-2 and Prx-4 might be larger than the other genes dectected in the BCP2, because the significance level was

higher than the other linkages and they were detected more times.

Linkages between isozyme markers and genes associated with the hypersensitive reaction using rate of necrosis development

Another criteria was used to detect the linkages with HR associated genes. It was the rate of necrosis development at each time interval. This idea was generated by studying the dynamic of necrosis development of H7998, F_1, LA 716, and each segregating population (Fig. 4.1 and Fig. 4.2). In a segregating population, whenever a gene associated with HR is expressed the phenotype of necrosis severity will be affected. Therefore, the rates of necrosis, Rh values (defined as hourly change of necrosis rating) between two consecutive time periods were used as variables in the linkage analyses. The advantages of using Rh values are : 1. the possibility to detect the HR associated genes with small effects were higher; and 2. the time period when the effect of each HR associated genes can be observed can be determined; 3. the detected linkage can be verified by the change of the order of the means between homozygotes and heterozygotes for each marker.

Because a large proportion of the plants had the same amount of increase in necrosis rating at any time period, the distribution was too discrete after ranking for the nonparametric analyses to be valid (Ott, 1988). Therefore, chi-square tests were used to test for the significant

difference of the frequency of rate between the genotypic classes of an enzyme locus. To apply this test, plants in each genotype of an enzyme locus were separated into two groups based on change (with Rh values larger than 0) or no change (with Rh values equal to 0) in necrosis ratings. This approach was used in order to reduce the percentage of the cells with small sample size (less than 5) and made the test more reliable. If more than 25% of the cells had sample size less than 5, the results of the chi-square test were ignored. In the backcrosses, the sample size of each genotype of an enzyme locus is large enough (greater than 30) to apply the central limit theorem (Ott, 1988). The means of the average rate were compared by a z test. However, in order to increase the efficiency, the z test was only applied in those cases where possible linkages were suggested by the chi-square tests.

In the backcross to the resistant parent (BCP1), significant differences in the frequency of rate (Rh values) were detected with 11 enzyme loci and one morphological locus at one or more time periods by the chi-square tests. Those loci in which significance was detected with only one time period were excluded from the possible linkages, because it was expected to detect significance at least twice (one with earlier and the other with later time periods) for a linked marker. Moreover, the order of the means of Rh values between homozygotes and heterozygotes for a linked marker was expected

to flip-flop. For example, if the homozygotes had faster rates earlier due to the effect of the linked gene, the rate of the heterozygotes would become faster later due to the susceptibility. Therefore, after determining the order of the means by z tests, five enzyme loci and one morphological locus were identified as the possible linked markers (Table 4.9). They were Prx-1, Skdh-1 on chromosome 1, Pgm-2 on chromosome 4, Got-2 on chromosome 7, Pn on chromosome 8, and Sod-1 on chromosome 11. The effects of the genes linked to Got-2 and Pn were observed during 0-16 hours period, Pgm-2 and Sod-1 during 16-24 period, and Prx-1 and Skdh-1 during 16-24 and 24-32 periods. When the effect of the above linked genes were observed, the homozygotes of Prx-1, Skdh-1, Got-2 and Pn had significantly higher rates than the heterozygotes. In contrast, heterozygotes of Pgm-2 and Sod-1 had significant higher rates than homozygotes. The significance levels of chi-square and z tests of the Got-2, Pn, and Sod-1 linkages were lower than the other linkages.

In the backcross to L. pennellii (BCP2), none of the linkages detected in the BCP1 was detected. However, two linkages were detected by the chi-square and z tests (Table 4.10). They were Est-7 on chromosome 2 and Prx-4 on chromosome 10. The effect of both linked genes was observed during 73-80 hours and the homozygotes of Est-7 had significantly higher rates than the heterozygotes and the heterozygotes of Prx-4 had significantly higher rates than the homozygotes.

In the F_2 population, only the chi-square test was performed, because the sample size of the homozygote groups was too small (less than 30) (Table 4.10) and the distribution was too discrete to be able to apply the central limit theorem (Ott, 1988). Thus, the results of F_2 were only used as confirmation of the findings in the reciprocal backcrosses. Only linkages with Prx-1 and Skdh-1 were detected in this population (Table 4.11). The effects of both the linked genes can be observed during 24 to 40 hours. During this period, confluent necrosis was observed in most of the plants of H7998.

Discussion

The polygenic nature of the hypersensitive reaction to cells of Xcv in plants of L. esculentum accession Hawaii 7998 was supported by linkages of isozyme markers to genes associated with the reaction. Furthermore, genes on more than one chromosome were found to affect the hypersensitive reaction. We are not aware of a similar mode of inheritance of the hypersensitive reaction in other plant-microbe interactions.

Many of the genes associated with HR in the resistant line to bacterial spot, Hawaii 7998, are possibly recessive. This was suggested by the fact that the phenotype of the F_1 plants was close to L. pennellii. Furthermore, the dynamic

change in necrosis development in the backcross to the susceptible parent (BCP2) was similar to that observed in the F_1 plants and the susceptible parent. The fastest necrosis development in the backcross to the resistant parent (BCP1) was observed at the same period when confluent necrosis was observed in the resistant parent. Therefore, the best chance to detect the linkages with the HR associated genes was to use the BCP1 population. Although most reported resistances associated with HR are controlled by a single dominant gene in the host, the recessive inheritance of HR has been reported in several other pathosystems (Crute, 1985).

Most of the linkages detected in the BCP1 backcross were not detected in the F_2 population by the chi-square test. Because of the small sample size in the F_2 population, the possibility of the combinations having the sample size less than 5 were increased and made most of the chi-square tests invalid. Also, most of the enzyme loci contained three genotypic classes, and the degree of freedom increased from 1 to 2. Thus, higher chi-square values were required for the same significance level. Thus, it was more difficult to detect linkages with genes with small effect in the F_2 population.

The linkages detected with Prx-1 and Skdh-1 are possibly due to the same gene. First, the two markers are linked on chromosome 1. Second, the homozygotes of H7998 allele for either markers had a significantly higher rate of necrosis

development. Third, the effect of both linked genes can be observed during the same time period. Since the linkage with Bnag-1 which is tightly linked to Skdh-1 was not detected, this implied that the linked gene is located between Prx-1 and Skdh-1 and possibly near to Prx-1.

The linkages detected in the BCP2 might be due to the genes associated with susceptibility, because the effect of these linked genes was observed at the same time when the fastest necrosis development was observed in the F_1 and susceptible parent. Klement (1982) suggested that the necrosis development of HR and susceptible reaction has similar processes, except that HR required a specific recognition and occurs soon after inoculation. The failure to detect these linkages in the BCP1 might be due to the significant skewing segregation of Est-7 and Prx-4.

Possibly, one major gene is directly involved in the mechanism of HR in Hawaii 7998 and the other genes modify or control the expression of the major gene without themselves being directly involved in the mechanism. This idea is supported by the fact that genes from L. pennellii had a significant effect on the HR reaction in progenies of the Hawaii 7998 - L. pennellii cross. The effect of the gene in the L. pennellii population cannot be determined, because plants were susceptible as determined by comparison with plants of the susceptible L. esculentum. Similarly, other genes with low significance that are involved in the polygenic

inheritance might only affect the intensity of the HR. Some isozyme marker-linked genes controlling the development of HR might encode positive or negative effects and shape the variation of HR in segregation population from discontinuous distinctive groups to continuous phenotypes. Whether the effects of the genes are additive for HR development in all cases has not been determined.

On the other hand, several independently-acting resistance genes could be involved and each gene alone could cause HR to strains of Xcv. This is possible because Hawaii 7998 may contain several resistance genes. For the gene-for-gene system to be operative, the strains of Xcv would have several independently-acting avirulence genes. If several gene-for-gene systems are operating in the Hawaii 7998-Xcv system, each gene-for-gene system could have a different intensity of HR development (Minsavage et al., 1990). In the plants of Hawaii 7998, only the gene-for-gene system which give the strongest HR would be recognized, but segregation system in the backcrosses or F2 population would then give the continuous nature of the reactions. There may or may not be different specificities of each gene-for-gene system to different strains of Xcv. We have not explored adequately the different specificities in the F_2 populations to differentiate strains of Xcv.

The Linkages detected used different criteria were very similar. The linkages detected with low significance level,

such as Got-2, Pn, and Sod-1, suggest that the linked genes may have small effects or they are far from the marker loci. This only can be determined when more surrounding markers (RFLP) are used and the Quantitative Trait Loci (QTL) likelihood map is developed in the future. In this study, only part of the tomato genome has been surveyed. Thus, it is possible that other genes associated with HR have not been detected. Other molecular markers, such as RFLP markers, can help the continuous survey the entire tomato genome and confirm the findings in this report.

Table 4.1 Frequency of plants of BCP1 progenies according to ratings for necrosis at different time periods.

Rating	Hours after infiltration										
	16	24	32	40	48	56	64	72	80	88	96
0	151	115	58	24	20	14	11	4	2	1	0
1	26	41	51	53	42	42	43	31	13	4	2
2	7	12	33	32	27	30	22	27	26	22	2
3	6	11	21	29	31	22	21	19	26	19	20
4	2	9	11	30	29	34	41	48	40	40	34
5	1	5	19	25	44	51	55	64	86	107	135
H7998	3.2[a] (0-4)[b]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
F1	0.0	0.0	0.1 (0-1)	0.1 (0-1)	0.5 (0-2)	0.5 (0-2)	0.8 (0-3)	1.1 (0-4)	1.8 (1-4)	2.8 (2-5)	4.0 (3-5)

[a] Average rating for necrosis of six plants of the parental types.
[b] Range of the necrosis ratings.

Table 4.2 Frequency of plants in BCP2 population according to ratings for necrosis at different time periods.

Rating	Hours after infiltration									
	40	48	56	64	73	80	88	96	104	112
0	157	137	113	88	35	12	5	2	0	0
1	18	36	52	71	66	53	41	15	7	5
2	1	2	7	10	41	24	21	22	9	2
3	0	0	2	2	16	24	13	14	9	7
4	0	1	1	1	10	30	18	14	23	4
5	0	0	1	4	8	33	78	109	128	158
F1	0.0[a]	0.1 (0-1)[b]	0.3 (0-1)	0.6 (0-1)	1.1 (0-3)	2.3 (1-5)	2.7 (1-5)	3.4 (2-5)	4.3 (3-5)	4.9 (4-5)
LA 716	0.0	0.0	0.1 (0-1)	0.3 (0-2)	1.0 (0-3)	1.4 (0-4)	2.1 (0-5)	3.0 (1-5)	3.9 (2-5)	5.0

[a] Average ratings for necrosis of seven plants of the parental types.
[b] Range of the necrosis ratings.

Table 4.3 Frequency of plants in F_2 population according to ratings for necrosis at different time periods.

Rating	Hours after infiltration													
	16	24	32	40	48	56	64	72	80	88	96	104	116	128
0	88	71	55	45	42	39	32	27	24	19	15	11	4	4
1	6	20	30	33	26	23	25	27	25	23	25	23	16	8
2	1	1	5	8	13	16	13	11	13	18	15	16	14	8
3	1	2	0	4	5	3	8	11	8	8	8	9	15	11
4	1	0	3	1	3	5	4	3	7	6	8	3	7	13
5	1	4	5	7	9	12	16	19	21	24	27	36	42	54
H7998														
	0.0[a]	0.9 0-2	2.9 1-5	4.3 3-5[b]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
F_1														
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1 0-1	1.0 0-2	2.1 1-5	3.2 1-5	4.4 3-5	5.0	5.0
LA 716														
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1 0-1	0.2 0-1	0.5 0-2	1.3 0-4	2.7 1-5	4.4 2-5

[a] Average ratings for necrosis of ten plants of prental types.
[b] Range of the necrosis ratings.

Table 4.4 Internal bacterial population of BCP1 progeny with different resistance level based on the rating of hypersensitive reaction.

Plant[a]	Days after infiltration					
	0	1	2	3	4	5
BB	3.58[b]A[c]	5.04 A	6.10 AB	7.19 AB	8.09 A	8.25 A
#103 S	3.56 A	4.94 AB	6.18 A	7.29 A	8.43 A	8.44 A
#70 I	3.55 A	4.89 AB	5.64 CD	6.69 C	7.34 B	7.46 B
#146 I	3.46 A	4.82 AB	5.91 ABC	6.76 BC	7.32 B	7.79 B
#42 I	3.53 A	4.76 B	5.84 ABC	6.63 C	7.45 B	7.81 B
#50 R	3.63 A	4.74 B	5.71 BCD	6.12 D	6.65 CD	6.19 D
#133 R	3.59 A	4.86 AB	5.60 CD	6.03 D	6.84 BC	6.82 C
#202 R	3.51 A	4.94 AB	5.40 D	6.32 CD	6.51 D	6.70 C
H7998	3.54 A	5.04 A	5.88 ABC	6.66 C	7.23 BC	7.48 B

[a] Bonny Best (BB) was used as susceptible control; Hawaii 7998 (H7998) was used as resistant control; the other plants were selected from BCP1 progeny with different resistant level (S: susceptible, I: intermediate, R: resistant).
[b] Average of three replications. The unit is log(cfu/cm^2).
[c] Multiple range Duncan test based on the data of the same column. Values with different letters were significantly different at the 0.05 level.

Table 4.5 Electrolyte leakage of BCP1 progeny with different resistant level based on the rating of hypersensitive reaction.

Plant	Hours after infiltration											
	0		12		24		36		48		60	
BB[a]	38[b]	B[c]	34.3	C	60	C	124.6	C	151	CD	197.6	CD
103S	53.7	AB	40.7	BC	66	C	88	C	114.3	D	111	D
70I	49.7	AB	44.7	ABC	80	C	133	C	242	C	290.6	BC
146I	43.7	AB	45.7	ABC	103	C	122.3	C	236.3	C	279.6	BC
42I	49.3	AB	48	ABC	80	C	149.3	C	339	B	322	B
50R	50	AB	45	ABC	154.6	BC	231	B	355	B	315.3	BC
133R	65.7	AB	39	BC	299.3	A	266.3	AB	383	B	335	B
202R	67.7	A	59.3	A	296.3	A	310	A	484.6	A	463.6	A
H7998	55.7	AB	53	AB	252.6	AB	246.3	AB	366	B	321	B

[a] BB (Bonny Best) was used as susceptible control; H7998 (Hawaii 7998) was used as resistant control; the other plants were selected from BCP1 progeny with different resistant level (S: susceptible, I: intermediate, R: resistant).
[b] Average of three replications. the unit is log(cfu/cm^2).
[c] Duncan multiple range Duncan test based on the data of the same column. Values with different letters were significantly different at the 0.05 level.

Table 4.6 Rank order correlation coefficients of necrotic ratings with electrolyte leakage (EL) and internal populations (POP), respectively. Data from the selected plants of BCP1 with different resistance levels.

	16	24	32	40	48	56	64	72	80	88	96
POP[a]	-0.73	-0.65	-0.83	-0.86	-0.88	-0.88	-0.82	-0.72	-0.77	-0.77	-0.73[c]
	NS	NS	**	**	**	**	**	*	*	*	*[d]
EL36[b]	0.86	0.91	0.91	0.85	0.90	0.90	0.88	0.88	0.82	0.82	0.84
	**	***	***	**	***	***	**	**	**	**	**

[a] Day 5 of internal bacterial population, data shown in Table 4.5.
[b] Data of electrolyte leakage at 36 hours shown in Table 4.6.
[c] Ranking correlation coefficient.
[d] Significant level of the p value under the hypothesis rho=0. NS means p less than 0.05; * means 0.05>=p>0.01; ** means 0.01>=p>0.001; *** means 0.001>=p>0.0001.

Table 4.7 Analysis of monogenic segregation of marker loci in BCP1 (Hawaii 7998x(Hawaii 7998xLA 716)), BCP2 ((Hawaii 7998xLA 716)xLA 716) and F_2 population. e/e esculentum homozygote; e/p pennellii heterozygote; p/p pennellii homozygote.

Locus	Chrom. No.	BCP1 No.plants e/e	BCP1 No.plants e/p	BCP1 X^2	BCP2 No.plants p/p	BCP2 No.plants e/p	BCP2 X^2	F_2 No. plants e/e	F_2 No. plants e/p	F_2 No. plants p/p	F_2 X^2
Prx-1	1	76	72	NS	102	73	4.8*	9	50	37	16.5***
Skdh-1	1	81	67	NS	88	72	NS	24	42	26	NS
Bnag-1	1	88	54	8.1**	-	-	-	21	46	25	NS
Est-7	2	120	73	11.5***	76	100	NS	27	53	18	NS
Prx-2	2	-	-	- [d]	-	-	-	28	51	18	NS
Prx-7	3	97	87	NS	70	57	NS	20	72[a]		NS
Pgm-2	4	93	97	NS	77	83	NS	26	48	23	NS
Prx-5	5	-	-	-	-	-	-		72	26[b]	NS
Aps-1	6	85	101	NS	70	80	NS	14	45	37	11.4**
Got-2	7	99	91	NS	80	88	NS	20	47	24	NS
Aps-2	8	99	75	NS	88	66	NS	28	46	21	NS
Pn [c]	8	74	96	NS	-	-	-	23	74[a]		NS
Est-2 [c]	9	106	77	4.6*	-	-	-	24	73[a]		NS
Prx-4	10	57	130	28.5***	71	68	NS	17	56	23	NS
Sod-1	11	69	118	12.8***	-	-	-	8	45	34	15.6***
Est-4	12	29	64	13.2***	86	72	NS	17	46	25	NS
6Pgdh-2	12	79	109	4.8*	-	-	-	18	54	26	NS
Pgi-1	12	77	115	7.5**	80	84	NS	16	62	20	8.2**

* significant difference at 0.05 level.
** significant difference at 0.01 level.
*** significant difference at 0.001 level.
[a] Genotypes were determined based on a unique band or phenotype of *L. pennellii*.
[b] Genotypes were determined based on a unique band of *L. esculentum*.
[c] Genotypes can not be determined in BCP2 due to the dominance of pennellii allele.
[d] Data were not be determined.

Table 4.8 The isozyme markers which showed significant linkage with ratings for necrosis of infiltrated areas of leaves in progenies at different time periods.

	Locus		Hours after infiltration													
			16	24	32	40	48	56	64	72	80	88	96	104	112	116	128
BCP1	Prx-1	1[a]	NS	**	****	****	****	****	****	***	*	**[b]					
	Skdh-1	1	NS	***	****	****	****	****	****	****	**	**					
	Pgm-2	4	NS	***	***	**	***	***	***	**	**	*					
	Got-2	7	*	NS	*	**	NS	NS	NS	NS	NS	NS					
	Pn	8	*	*	NS	NS	NS	NS	NS	NS	NS	NS					
	Prx-4	10	NS	NS	NS	*	*	NS	NS	NS	*	*					
	Sod-1	11	NS	*	NS	NS	NS	NS	NS	NS	NS	NS					
BCP2	Est-7	2					NS	NS	NS	NS	NS	*	*	**	*		
	Pgm-2	4					NS	NS	*	NS	NS	NS	NS	NS	NS		
	Aps-2	8					**	**	**	**	*	*	*	*	*		
	Prx-4	10					NS	NS	*	**	**	**	**	NS	NS		
	Pgi-1	12					NS	NS	NS	NS	NS	NS	*	*	NS		
F_2	Prx-1	1	NS	NS	*	**	**	**	**	**	**	**	**	*		NS	NS
	Skdh-1	1	NS	NS	*	***	**	***	***	***	***	***	***	***		**	*
	Bnag-1	1	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS		NS	NS
	Est-7	2	***	***	***	**	**	**	*	*	*	*	NS	NS		NS	NS
	Prx-2	2	**	**	***	**	**	**	**	**	**	*	*	NS		NS	NS
	Pgm-2	4	NS	NS	*	*	*	NS	NS	*	*	*	*	**		*	NS
	Prx-5	5	*	NS	*	*	**	*	NS	NS	NS	**	**	**		*	NS
	Est-2	9	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*		*	*
	Prx-4	10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	*

[a] Chromosome number for each marker.
[b] Significance level of the nonparamatric analysis: NS:nonsignificant, p value greater than 0.05; * :0.01<p<=0.05; ** : 0.001<p<=0.01; *** : 0.0001<p<=0.001; **** : p<=0.0001.

Table 4.9 The possible linkages with the genes associated with hypersensitivity detected in the backcross to the resistant paren∫√∩xxytxhBCP1).

		Rh [a] 0-16	Rh 16-24	Rh 24-32	Rh 40-48	Rh 64-72	Rh 72-80
Prx-1	e/e [b]		8.2	13.2		2.6	3.6
	e/p [b]		2.6	7.3		5.7	8.3
	X^2 [c]		12.1 D[e]	5.2 A		8.3 B	14.6 D
	Z [d]		3.9 D[e]	3.6 C		-3.1 C	-3.4 C
Skdh-1	e/e		8.2	12.8		2.9	3.7
	e/p		2.6	7.3		5.6	6.0
	X^2		19.0 D	7.0 B		5.7 A	14.4 D
	Z		4.1 D	3.4 C		-2.6 B	-4.4 D
Pgm-2	e/e		3.9				6.7
	e/p		7.6				3.7
	X^2		14.6 D				5.6 A
	Z		-2.6 B				2.5 B
Got-2	e/e	3.0			3.9		
	e/p	1.4			6.0		
	X^2	4.0 A			5.8 A		
	Z	2.1 A			-2.2 A		
Pn	e/e	3.6				3.2	
	e/p	1.6				5.1	
	X^2	6.7 B				4.7 A	
	Z	2.0 A				-1.8 A	
Sod-1	e/e		4.0				8.2
	e/p		7.0				3.5
	X^2		4.9 A				11.9 C
	Z		-2.1 A				3.5 C

[a] The rate of necrosis development (increase of rating x100/hour).
[b] The average of the Rh values of the homozygotes (e/e) and the heterozygotes (e/p).
[c] Chi-square value of the independence test which tested the similarity of the frequency distribution between the genotypes of a segregating enzyme locus. For this test, the plants were separated into two groups, one with no increase in necrosis rating and the other with increase.
[d] The Z value of the comparison between the mean rate of different genotypes.
[e] The significant level of the chi-square test or Z test. A : 0.05 level, B : 0.01 level, C : 0.001 level, and D : 0.0001 level.

Table 4.10 The possible linkages with the genes associated with hypersensitivity detected in the backcross to the susceptible parent (BCP2).

				Rh [a] 73-80		Rh 96-104
Est-7	p/p [b]	17.5			3.5	
	e/p [b]		12.6			7.6
	X^2 [c]		6.5 A [e]			6.4 A
	Z [d]		2.6 B [e]			-3.3 C
Prx-4	p/p		12.7			7.9
	e/p			18.1		3.9
	X^2			4.2 A		9.2 B
	Z			-2.5 B		2.6 B

[a] The rate of necrosis development (increase of rating x100/hour).
[b] The average of the Rh values of the homozygotes (e/e) and the heterozygotes (e/p).
c chi-square value of the independence test which was tested the similarity of the frequency distribution between the genotypes of an segregating enzyme locus. For this test, the plants were separated into two groups, one with no increase in necrosis rating and the other with increase.
[d] The Z value of the comparison between the mean rate of different genotypes.
[e] The significant level of the chi-square test or Z test. A : 0.05 level, B : 0.01 level, C : 0.001 level, and D : 0.0001 level.

Table 4.11 The possible linkages with the genes associated with hypersensitivity detected in the F_2 population.

	Rh_{24-32}[a]	Rh_{32-40}	Rh_{40-48}	$Rh_{116-128}$
Prx-1	7.3[b] A[c]		8.8 A	6.4 A
Skdh-1	6.7 A	7.6 A		6.4 A

[a] The rate of necrosis development (increase of rating x100/hour).
[b] Chi-square value of the independence test which was tested the similarity of the frequency distribution between the genotypes of an segregating enzyme locus. For this test, the plants were separated into two groups, one with no increase in necrosis rating and the other with increase.
[c] The significant level of the chi-square test. A : 0.05 level.

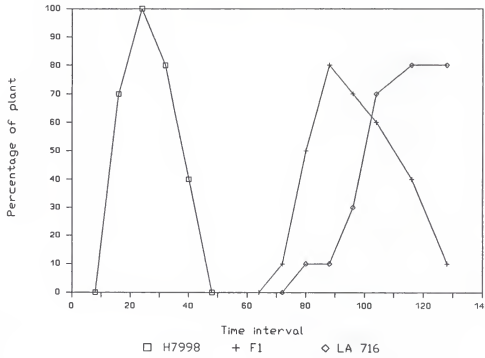

Figure 4.1 Dynamic of necrosis development of H7998, the F_1, and LA 716. Data used to create this figure was the parental control of F_2 population experiment.

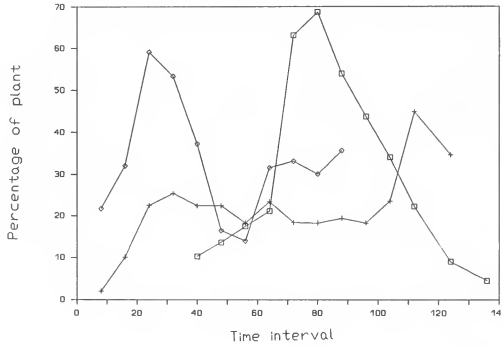

Figure 4.2 Dynamic of necrosis development of the backcross to H7998, backcross to LA 716 and F_2 populations.

CHAPTER 5
RELATIONSHIPS OF FIELD RESISTANCE AND THE INTENSITY OF HYPERSENSITIVITY

Introduction

Hypersensitive reaction (HR) is a defense mechanism of the plant accompanied by rapid tissue necrosis (Klement, 1982). Often, phytoalexin accumulates in the incompatible host-parasite interaction (Long et al., 1985). However, it has been debated whether or not HR has a primary role in resistance. A tomato accession of Lycopersicum esculentum Mill, Hawaii 7998 (H7998), is the only reported source of resistance to bacterial spot caused by Xanthomonas campestris pv. vesicatoria (Xcv) (Scott and Jones, 1986) and HR has been identified in the resistant line induced by Xcv (Jones and Scott, 1986). Usually, hypersensitivity is described as a form of specific or vertical resistance, and is monogenically inheritable (Crute, 1985). However, inheritance of Xcv resistance in H7998 is quantitative under field conditions (Scott and Jones, 1989).

The objectives of this chapter were (1) to determine the relationships between HR intensity and field resistance and (2) to study the linkages of field resistance in H7998 with isozyme markers.

Materials and Methods

Plant materials

Two crosses were made for these studies. First, plants of Lycopersicon esculentum Mill. accession Hawaii 7998 (H7998) and a susceptible breeding line Fla. 7060 (7060) was used to produce seeds of F_2 population. The population of 100 plants used here was the same as that in chapter 3. Second, H7998 and the wild species L. pennellii accession LA 716 were used to produce seeds of an interspecific F_1 hybrid and backcross to the resistant parent (H7998 x [H7998 x LA 716], BCP1). The population of 193 plants used in this chapter was the same as that used in Chapter 4.

Isozyme analysis

The population used in this analysis was the backcross to H7998 of the H7998-LA 716 cross. The isozyme data were presented in Chapter 4. The genotypes of the following isozyme loci were determined : Prx-1, Skdh-1 and Bnaq-1 on chromosome 1; Est-7 on chromosome 2; Prx-7 on chromosome 3; Pgm-2 on chromosome 4; Aps-1 on chromosome 6; Got-2 on chromosome 7; Aps-2 on chromosome 8; Est-2 on chromosome 9; Prx-4 on chromosome 10; Sod-1 on chromosome 11; Est-4, 6Pgdh-2 and Pgi-1 on chromosome 12. One morphological locus Pn on chromosome 8 was also examined.

Evaluation of hypersensitivity and field resistance

Two populations of the different crosses were studied at different seasons. For both populations, the tested plants were first evaluated the development of necrosis in the growth room or growth chamber, which have been described in Chapter 3 and 4. The same plants were then transplanted into the field for the observation of field resistance. The field tests of the F_2 population was conducted in summer of 1989 at Bradenton, Florida. Plants of H7998 and 7060 were used as controls. The field tests of the BCP1 was conducted in summer of 1990 at Bradenton, Florida. Plants of H7998 and F_1 were used as controls. Plants were transplanted on beds 20 cm high, 76 cm wide, and with 137-cm centers. Plants were spaced 61 cm apart within rows. Plants were watered by seepage irrigation and normal insect control and fertilizer practices were used. Plants were staked and tied. Plants were inoculated with a bacterial suspension (10^8 cfu/ml) misted on the plants in the early mornings. Each plant was rated for disease severity using the Horsfall-Barratt Scale (Horsfall and Barratt, 1945).

Statistical analysis

The data sets of the field rating for the backcross to H7998 (BCP1) on September 6 was designated as FD1 and on September 21 as FD2. Plants with similar field resistance

rating as the resistance parent were used to generate new data sets, FD1r from FD1 and FD2r from FD2, respectively. The relationships among ratings of necrosis after artificial inoculation and field resistance were determined by the rank order correlation coefficient which measures the monotonic association between variables. This analysis was used because a linear association among these three variables does not exist.

For linkage analysis, the ratings of field resistance of the BCP1 population were converted into percentage of diseased area following the conversion table (Redman et al., 1968). After conversion, the distribution of field resistance was assumed to be normal. Thus, the means of each genotypes for a segregating enzyme marker were compared by a t test. A two-way F test was used to test for the equality of the variances of the two genotypes. If the variances were significantly different, a separate-variance t test was used (Ott 1988). All statistical analyses were performed on a PC computer using the Statistical Analysis System (SAS Institute, SAS Circle, Box 8000, Cary, North Carolina).

Results and Discussion

Ratings of field resistance

In the field test of the F_2 population of the cross between H7998 and 7060, all the plants of H7998 had a rating

of 2. The average rating for 7060 was 4.8 and ranged from 3 to 5. In the F_2 population, 18 plants of the total population with a rating of 2, 40 plants with a rating of 3, 30 plants with a rating of 4, 10 plants with a rating of 5, and 1 plant with a rating of 6.

In the field test for the BCP1 of the cross between H7998 and LA 716, the average disease severity of H7998 was 1.2 on September 6 and 1.3 on September 21, and both ranged from 1 to 2. The average severity of F_1 plants was 3.8, and ranged from 3 to 4 both on September 6 and on September 21. Continuous variation in field resistance was observed on both dates (Fig. 5.1 and 5.2). Disease pressure was greater on September 21, because frequency distribution skewed to the lower rating side on September 6. The field experiment was abandoned due to the severe occurrence of viral diseases and fungal diseases, mainly early blight.

Correlations between HR and field resistance

The BCP1 population data of the H7998-LA 716 cross were used in this study. The correlations between each necrosis ratings and field resistance were significant at least at the 0.05 level when the data set of FD1 or FD2 was used in the statistical analysis. Correlation coefficients tended to be higher using the FD2 data (Table 5.1). The highest correlation coefficients were detected between the necrosis ratings at 40 and 48 hours and both FD1 and FD2.

The correlation between FD1r and the necrosis ratings was not significant. This might be due to the lower disease pressure on September 6. Thus, some of the plants with lower rating of field resistance might not be truly resistant. The correlation between FD2r and the ratings of necrosis at 32 and 40 hours were significant at the 0.05 level (Table 5.1). During this period, a fast development of necrosis was occurring which was indicated by the highest percentage of plants with an increase in necrosis ratings (Table 5.1).

Relationships between HR and field resistance

In the BCP1 of the H7998-LA 716 cross, the necrosis rating at 40 hours (NR40) after infiltration was used to further explore the relationships between HR and field resistance. The reasons to use NR40 were : 1) at this time, all plants of H7998 had confluent necrosis symptom; 2) judging by the dynamic of necrosis development in the population (Fig. 4.2), the effect of HR have expressed. A contingency table was created between NR40 and FD2 (Table 5.2). All the plants that showed high field resistance (Horsfall-Barratt scale=1) had strong HR (NR40=5). The frequency of those plants sharing the same field rating (FD2) according to necrosis rating was indicated by row percentage. When the field resistance

decreased, the frequency distributions continued skewing to the '0' side which indicated that less of the plants had strong HR (Fig. 5.3). The percentage of those plants with necrosis rating of '5' to be resistant in the field (with Horsfall-Barratt scale of '1' or '2') was 54.5%. The frequency of those plants sharing the same NR40 according to the field rating was indicated by column percentage (Table 5.2). The frequency distribution was skewed to the resistant side (lower rating for field resistance) in the group of plants with NR40 equal to 5 (Fig. 5.4). When the necrosis rating decreased, the distribution continued skewing to the right side which indicated that less plants had high field resistance.

In the F_2 of the H7998-7060 cross, the rating of necrosis at 60 hours (NR60) after infiltration was used to study the relationship with field resistance. The reason to use NR60 was that all plants of H7998 showed confluent necrosis symptom at this time. A contingency table between NR60 and field rating was established (Table 5.3). Similar relationships between HR and field resistance were observed in this population. The percentage of those plants had necrosis rating of '5' to be resistant in the field (with Horsfall-Barratt scale of '2') was 29.1%.

The studies of the relationships between HR and field resistance indicated that HR is not the only mechanisms for the field resistance, because not all the plants showed strong

field resistance had strong HR. However, HR did play an important role for the field resistance, because about 50% of plants selected by strong HR in the backcross population also showed strong field resistance. Therefore, to select the plants based on HR is still a quite efficient method for resistance breeding.

Linkages between isozyme markers and field resistance

Fifteen isozyme markers and one morphological marker were used in the linkage analyses using the BCP1 population (Table 5.4). Linkages of isozymes and field resistance detected with the FD1 data set were Prx-1 on chromosome 1, Est-2 on chromosome 9, and Est-4 on chromosome 12. The homozygotes of Prx-1 had significantly lower disease severity than the heterozygotes. In contrast, heterozygotes of Est-2 and Est-4 had significantly lower disease severity. The linkages with Prx-1 and Est-2 were detected again with the FD2 data set. Two additional linkages were detected with the FD2 data set. They were Skdh-1 and Bnag-1 on chromosome 1. In both cases, the homozygotes had significantly lower disease severity than the heterozygotes.

Since disease pressure was higher on September 21, the linkages detected with FD2 should be more reliable. The linkages with Prx-1 and Skdh-1 were also associated with HR (results presented in chapter 4). This is further evidence that field resistance is associated with HR. The Est-2 linked

gene might control another resistance mechanism, because it was not associated with HR (results presented in chapter 4). Due to variation of the environment, it would be appropriate to repeat the field experiment to confirm the above findings.

Table 5.1 Rank order correlation coefficients between rating of field resistance and the rating of necrosis.

Time	FD1[a]	FD2[a]	FD1r[b]	FD2r[b]	% of plant[c]
16	-0.16[d]*[e]	-0.16 *	-0.24	-0.36	21.7
24	-0.19 *	-0.30 ****	-0.22	-0.48	32.1
32	-0.34 ****	-0.39 ****	-0.03	-0.50 *	59.1
40	-0.39 ****	-0.41 ****	-0.04	-0.46 *	53.3
48	-0.39 ****	-0.41 ****	-0.10	-0.32	37.3
56	-0.36 ****	-0.39 ****	-0.08	-0.33	16.5
64	-0.36 ****	-0.39 ****	-0.06	-0.30	14.0
72	-0.35 ****	-0.39 ****	-0.13	-0.27	31.6
80	-0.35 ****	-0.33 ****	-0.10	-0.27	33.2
88	-0.28 ***	-0.33 ****	0.13	-0.20	30.1
96	-0.29 ***	-0.30 ****	0.20	-0.11	35.7

[a] FD1 : data set of field resistance recorded on September 6 for the entire tested plants. FD2 : data set of field resistance recorded on September 21 for the entire tested plants.
[b] The data sets FD1r and FD2r were generated from FD1 and FD2, respectively, in which the plants had similar ratings of field resistance as the resistance parent.
[c] The percentage of plants with increase in rating of necrosis during the time from the previous rating to the current rating.
[d] Rank correlation coefficients between the field resistance and the rating of necrosis at different times.
[e] The significance level under Ho: Rho=0, *: 0.05 level, **: 0.01 level, ***: 0.001 level, ****: 0.0001 level.

Table 5.2 Two-way contingency table between ratings of field resistance recorded on September 21 (FD2) and ratings of necrosis at 40 hours after infiltration (NR40). The population used in this study was the backcross to Hawaii 7998 of the cross between Hawaii 7998 and LA 716.

		NR40					
FD2		0	1	2	3	4	5[a]
1[b]	Frequency	0[c]	0	0	0	0	5
	Row %	0.0	0.0	0.0	0.0	0.0	100.0
	Column %	0.0	0.0	0.0	0.0	0.0	22.7
2	Frequency	1	3	2	1	7	7
	Row %	4.8	14.3	9.5	4.8	33.3	33.3
	Column %	5.3	7.1	7.7	3.7	30.4	31.8
3	Frequency	3	9	8	11	6	6
	Row %	7.0	20.9	18.6	25.6	14.0	14.0
	Column %	15.8	21.4	30.8	40.7	26.1	27.3
4	Frequency	8	18	11	11	5	4
	Row %	14.0	31.6	19.3	19.3	8.8	7.0
	Column %	42.1	42.9	42.3	40.7	21.7	18.2
5	Frequency	6	10	5	3	4	0
	Row %	21.4	35.7	17.9	10.7	14.3	0.0
	Column %	31.6	23.8	19.2	11.1	17.4	0.0
6	Frequency	1	2	0	1	1	1
	Row %	20.0	40.0	0.0	20.0	20.0	20.0
	Column %	5.3	4.8	0.0	3.7	4.4	0.0

[a] Necrosis rating scale.
[b] Field resistance rating based on Horsfall-Barratt scale.
[c] The number of plants which had the NR40=0 and FD2=1.

Table 5.3 Two-way contingency table between ratings of field resistance recorded on (FD) and ratings of necrosis at 60 hours after infiltration (NR60). The population used in this study was the F_2 of the cross between Hawaii 7998 and a susceptible breeding line 7060.

		NR60					
FD		0	1	2	3	4	5[a]
2[b]	Frequency	0[c]	0	2	6	3	7
	Row %	0.0	0.0	11.1	33.3	16.7	38.9
	Column %	0.0	0.0	11.1	25.0	17.7	29.2
3	Frequency	0	6	5	11	9	9
	Row %	0.0	15.0	12.5	27.5	22.5	22.5
	Column %	0.0	42.9	27.8	45.8	52.9	37.5
4	Frequency	1	4	8	5	4	7
	Row %	3.5	13.8	27.6	17.2	13.8	24.1
	Column %	100.0	28.6	44.4	20.8	23.5	29.2
5	Frequency	0	3	3	2	1	1
	Row %	0.0	30.0	30.0	20.0	10.0	10.0
	Column %	0.0	21.4	16.7	8.3	5.9	4.2
6	Frequency	0	1	0	0	0	0
	Row %	0.0	100.0	0.0	0.0	0.0	0.0
	Column %	0.0	7.1	0.0	0.0	0.0	0.0

[a] Necrosis rating scale.
[b] Field resistance rating based on Horsfall-Barratt scale.
[c] The number of plants which had the NR60=0 and FD=2.

Table 5.4 Linkage relationships between isozyme marker loci and field resistance in the backcross to the resistance parent, Hawaii 7998. e/e, esculentum homozygote; e/p, pennellii heterozygote.

Locus	Chromosome no.		No. of plants e/e	e/p	X^2	Mean of disease[a] e/e	e/p	t	
Prx-1	1	FD1[b]	78	78	0.0	3.4	5.7	-3.0	**[c]
		FD2[b]	77	77	0.0	7.6	10.7	-2.5	*
Skdh-1	1	FD1	73	54	2.8	3.8	5.4	-1.8	
		FD2	72	53	2.9	7.3	12.4	-3.7	***
Bnag-1	1	FD1	76	44	8.5 **	3.8	5.3	-1.9	
		FD2	75	43	8.7 **	7.7	12.5	-3.2	**
Est-7	2	FD1	102	59	11.5 ***	4.4	4.6	-0.3	
		FD2	100	59	10.6 ***	9.4	8.5	0.7	
Prx-7	3	FD1	81	74	0.3	4.1	5.0	-1.1	
		FD2	79	74	0.2	8.2	10.2	-1.6	
Pgm-2	4	FD1	75	84	0.5	4.2	4.7	-0.7	
		FD2	74	83	0.5	8.2	10.0	-1.5	
Aps-1	6	FD1	77	81	0.1	4.1	4.9	-1.0	
		FD2	75	81	0.2	8.3	10.0	-1.4	
Got-2	7	FD1	81	78	0.1	5.0	3.9	1.4	
		FD2	81	76	0.2	9.1	9.2	-0.0	
Aps-2	8	FD1	83	67	1.7	4.5	3.9	0.9	
		FD2	81	67	1.3	9.3	8.5	0.6	
Pn	8	FD1	59	81	3.5	4.8	4.2	0.7	
		FD2	58	80	3.5	9.7	8.9	0.6	
Est-2	9	FD1	92	64	5.0 *	5.3	3.2	3.0	**
		FD2	91	63	5.1 *	10.0	7.6	2.1	*
Prx-4	10	FD1	46	112	27.6 ***	4.8	4.4	0.4	
		FD2	44	112	29.6 ***	8.9	9.2	-0.2	

Table 5.4--continued

Locus	Chromosome no.		No. of plants e/e	e/p	X^2		Mean of disease[a] e/e	e/p	t	
Sod-1	11	FD1[b]	56	100	12.4	***	4.5	4.3	0.2	
		FD2[b]	56	98	11.4	***	10.2	8.6	1.3	
Est-4	12	FD1	29	53	7.0	**	5.9	3.7	2.1	*[c]
		FD2	29	53	7.0	**	10.9	8.5	1.3	
6Pgdh-2	12	FD1	67	90	3.4		4.9	4.3	0.7	
		FD2	65	90	4.0	*	9.9	8.7	1.0	
Pgi-1	12	FD1	67	93	4.2	*	4.5	4.3	0.2	
		FD2	65	93	5.0	*	9.7	8.6	0.8	

[a] The percentage of diseased area.
[b] FD1 : data set of field resistance recorded on 6 Sept. for the entire tested plants. FD2 : data set of field resistance recorded on 21 Sept. for the entire tested plants.
[c] The significance level, *: 0.05 level, **: 0.01 level, ***: 0.001 level, ****: 0.0001 level.

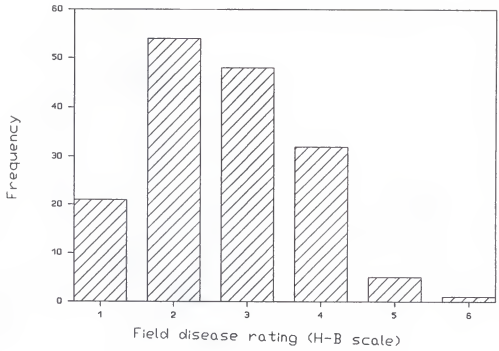

Figure 5.1 Frequency distribution of plants in the backcross to the resistance parent, Hawaii 7998 based on the rating of field resistance on 6 Sept. 1990. Horsfall-Barratt (H-B) scale was used for the rating. The average rating for the resistance parent was 1.2 (range from 1 to 2) and 3.8 (range from 3 to 4) for the F_1 plants.

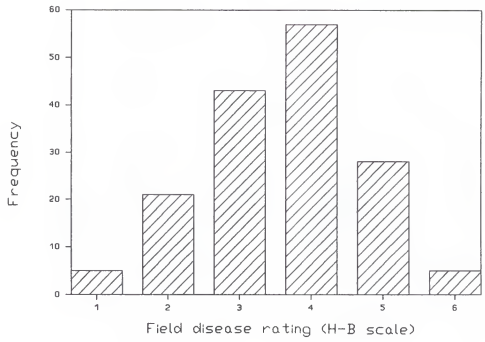

Figure 5.2 Frequency distribution of plants in the backcross to the resistance parent, Hawaii 7998 based on the rating of field resistance on 21 Sept. 1990. Horsfall-Barratt (H-B) scale was used for the rating. The average rating for the resistance parent was 1.3 (range from 1 to 2) and 3.8 (range from 3 to 4) for the F_1 plants.

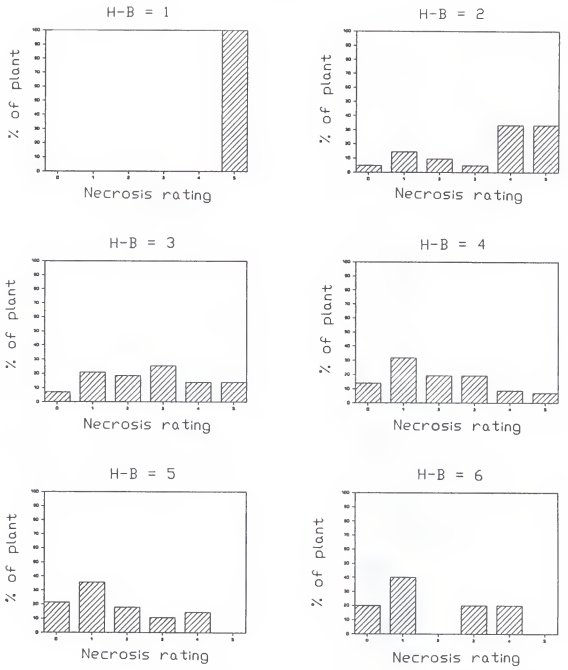

Figure 5.3 Frequency distribution of plants with the same ratings of field resistance (Horsfall-Barratt scale, H-B) according to the ratings of necrosis at 40 hours after inoculation. The data used to create this figure was in Table 5.2.

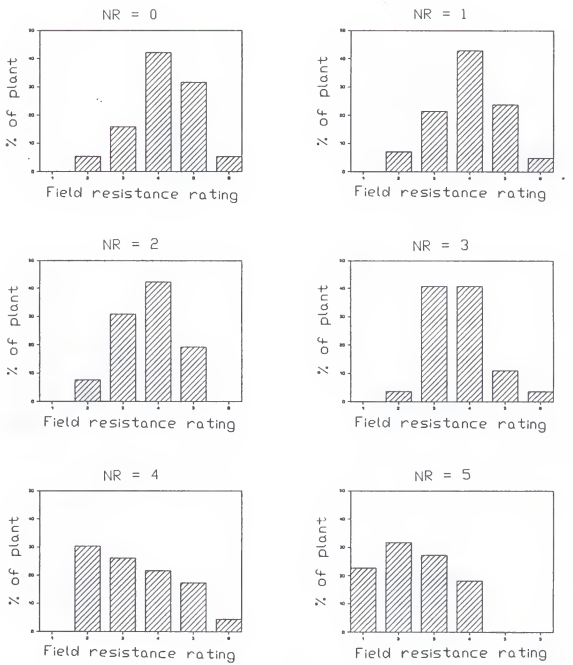

Figure 5.4 Frequency distribution of plants with the same ratings of necrosis at 40 hours after infiltration (NR40) according to the ratings of field resistance. The data used to create this figure was in Table 5.2.

CHAPTER 6
A NEW RACE OF XCV ON TOMATO AND SCREENING TOMATO GENOTYPES FOR RESISTANCE TO THIS NEW RACE

Introduction

Bacterial spot in tomato caused by Xanthomonas campestris pv. vesicatoria (Xcv) is a major problem in tomato production (Pohronezny et al., 1986). An accession of tomato, Hawaii 7998 (H7998), is reported to be resistant to strains of Xcv in Florida and other areas of the United States (Scott and Jones, 1986; Jones and Scott, 1986). However, it was reported that this resistant line is susceptible to the local strains of Xcv in Brazil (Nagai and Sugimori, 1986).

The causal bacterium can be classified into three groups based on the virulence to tomato and pepper. The tomato group (XcvT) is virulent on tomato only, the pepper group on pepper only (XcvP), and the pepper-tomato group on tomato and pepper (XcvPT). Within the XcvP and XcvPT groups, races of the pathogen can be distinguished by their disease reaction patterns on a set of near-isogeneic lines of pepper. The near-isogeneic lines were derived from Early Calwonder (ECW, a susceptible pepper cultivar to Xcv); ECW-10R, ECW-20R, and ECW-30R contain the resistance genes, Bs1, Bs2, and Bs3, respectively (Minsavage et al., 1990).

The objectives of this chapter were: 1) to determine whether the strain from Brazil is a new race of Xcv based on the response on a set of differential host plants and the ability to cause hypersensitive reaction (HR) in H7998; 2) to screen tomato genotypes for resistance to the Brazilian strain.

Materials and Methods

Bacterial strains and culture conditions

Strains of Xcv were stored in sterile tap-water. Strains were subcultured on nutrient agar and transferred to nutrient broth and grown overnight on a rotary shaker. Bacterial suspensions were prepared by centrifugation of overnight cultures, resuspended in sterile tap-water, and standardized to an absorbance of 0.3 at a wavelength of 600 nm (concentration about 5×10^8 cfu/ml).

The strain from Brazil, XV56, was obtained from Dr. J. B. Jones, Gulf Coast Research and Education Center, Bradenton FL, for comparisons with strains 75-3 (XcvT), 82-8 (XcvP race 1), E3 (XcvPT race 2), and 88-5 (XcvPT race 3). Strain 82-8uns, a mutant derived from 82-8 that lost the ability to induce HR on ECW-20R and ECW-30R (Minsavage et al., 1990), was used as a recipient for the cosmid clone pST103 in the conjugation, which is a subclone from pEC103. pEC103 is a pLAFR3 clone from strain 75-3 with *avrBsT* activity. The avirulence gene

avrBsT controls the ability of the XcvT group to induce HR on pepper (Minsavage et al., 1990).

Race identification

Suspensions (5 x 10^8 cfu/ml) of the strains XV56 and 75-3 were infiltrated into leaves of a set of differential plants, which included ECW, ECW-10R, ECW-20R, ECW-30R, Hawaii 7998 (H7998, resistant line of tomato to Xcv), and Bonny Best (BB, a tomato cultivar susceptible to Xcv). After infiltration, tomato plants were placed in a growth chamber with a 16 hr-day 8 hr-night cycle at 24°C and pepper plants were placed in the greenhouse with the temperature ranging from 20 to 35°C. The time for appearance of confluent necrosis in each plant was recorded in order to determine the classification status of XV56.

Verification of the HR was determined by comparing electrolyte leakage patterns in leaf tissues of the two tomato lines inoculated with XV56 and 75-3 at an inoculum concentration of 5 x 10^8 cfu/ml. Plants were incubated at 24°C in a growth chamber with a 16 hr-day 8 hr-night cycle. Leaf samples were collected every 12 hours after infiltration and the conductivity of water containing inoculated tissues was determined according to the procedure reported by Hibberd et al. (1987b). Three leaflets from the same plants were used as replications.

Further verification of the HR was confirmed by growth curves of XV56 and 75-3 in leaves of plants of H7998 and BB. The inoculum was adjusted to about 10^5 cfu/ml. Plants were incubated at 24°C in a growth chamber with a 16 hr-day 8 hr-night cycle after infiltration and leaf samples were taken daily for 6 days. Bacterial populations were determined by a standard dilution-plate method. Three leaflets from the same plant were used as replications.

Hypersensitive reaction in the hybrid of Solanum lycopersicoides and Lycopersicon esculentum

In a preliminary test, cells of XV56 caused HR in F_1 plants of the cross of Lycopersicon esculentum (VF36) and Solanum lycopersicoides. In order to understand the source of resistance, cells (5 x 10^8 cfu/ml) of 75-3, XV56, E-3, 82-8uns, and 82-8uns containing pST103 were infiltrated in leaves of the Solanum parent, the hybrid, BB, and ECW. Bonny Best was used as a substitute for VF36 to test the reaction of a Lycopersicon parent, and ECW was used to test the existence of the avrBsT activity. All tested plants were placed in the growth chamber at 24°C after infiltration. The time for appearance of confluent necrosis was recorded.

Screening Lycopersicon genotypes for resistance to XV56

A collection of 219 tomato lines which included some common cultivars, breeding lines, and PI lines was provided by

Dr. J. W. Scott, Gulf Coast Research and Education Center, Bradenton, FL. Four different inoculation methods were used in screening for resistance to XV56. These methods were : 1) leaflets of plants were infiltrated with a bacterial suspension (5×10^8 cfu/ml) and the plants were incubated at 24 C growth chamber. The appearance of confluent necrosis was recorded every 24 hours; 2) plants were incubated at 24 C in a growth chamber for 4 days and then leaflets were infiltrated with a bacterial suspension (5×10^8 cfu/ml). The plants were then moved to the greenhouse for the observation of confluent necrosis; 3) a bacterial suspension (5×10^8 cfu/ml) containing a small amount of carborundum was gently rubbed on the upper surface of young leaves with a cotton swab. Plants were incubated at 24°C in a growth chamber for 4 days and then moved to the greenhouse. The lesion sizes of each tested line were compared to those of H7998 and BB; and 4) 2 week-old plants were dipped in a bacterial suspension (10^6 cfu/ml) containing 0.025% (v/v) of surfactant L-77 (Union Carbide, Danbury, CT 06817-0001). Only 103 of the 219 tomato lines were tested with this method. Plants were incubated in the greenhouse for lesion size assessment.

Plants for the above inoculations were grown in the greenhouse. The same plants were used for methods 1, 2, and 3. For each method, 2 to 3 plants were used as replications and 20 to 25 lines were tested at the same time. Plants of H7998 and BB were used as standards for each inoculation.

Results and Discussion

Race identification

The reactions of the previously described pathogenic groups and races of Xcv are reported in Table 6.1. The responses of XV56 in the differential plants were different from the previously reported races of Xcv. This strain caused a light-brown, non-confluent necrosis within 24 to 36 hours in all pepper lines and the responses were similar to that for 75-3. Thus, XV56 belongs to the tomato group of Xcv strains (XcvT). XV56 differed from 75-3 in being virulent on plants of H7998.

Data from electrolyte leakage and internal bacterial growth patternsfurther determined that XV56 was virulent in leaves of H7998. An increase in electrolyte leakage in plants of H7998 infiltrated with 75-3 appeared 12 hours after infiltration with a rapid increase occurring between 12 hours and 24 hours which almost reached the maximum level (Fig. 6.1). The changes in electrolyte leakage were similar in H7998 infiltrated with XV56 and in BB infiltrated with either strain. The leakage of electrolytes in those treatments did not increased much from 0 to 36 hr after infiltration, but increased thereafter. The population of 75-3 in H7998 increased from 7.3×10^3 to 7.8×10^5 cfu/cm^2 2 days after infiltration (Figure 6.2). After 2 days, the population remained at the

same level. In BB, the population of 75-3 started at 7.7 x 10^3 cfu/cm^2 and reached the maximum of 1.4 x 10^7 cfu/cm^2 3 days after infiltration. However, the population patterns of XV56 in H7998 and BB were very similar. The populations started at 6 x 10^3 and 5.5 x 10^3 cfu/cm^2 in H7998 and BB, respectively, and continuously increased to the level of 10^8 cfu/cm^2 5 days after infiltration.

Three characteristics commonly used to distinguish the HR from a compatible reaction are rapid development of confluent necrosis when infiltrated with high concentrations of bacteria, rapid increase in electrolyte leakage shortly after infiltration, and lower population development after infiltration (Klement, 1982). These three characteristics of HR were not observed in the interaction of XV56 and H7998 which distinguished XV56 from 75-3. Thus, XV56 is designated as race 2 of the strains of the XcvT group. In the future, H7998 can be used as a differential host to distinguish race 1 of XcvT, such as 75-3, and race 2 of XcvT, such as XV56.

Currently, H7998 is the only reported resistance line to bacterial spot of tomato. Although strains similar to XV56 have seldom been isolated in the United States, this finding should alert tomato breeders that a new race of Xcv needs to be considered. Further research needs to be done such as determining the global distribution of this new race and identifying characteristics of this race which may aid in its detection.

Hypersensitive reaction in the hybrid of Solanum lycopersicoides and Lycopersicon esculentum

Cells of both XV56 and 75-3 were found to be incompatible in F_1 plants of the cross of S. lycopersicoides with L. esculentum 'VF36' (Table 6.2). Confluent necrosis was observed from 16 to 24 hr after infiltration of leaves with suspensions (5×10^8 cfu/ml) and incubated at 24 C. Confluent necrosis in compatible plants occurred after 36 hours. Cells of E-3, race 2 of the pepper/tomato group of strains appeared to cause a compatible reaction in all plants.

Since XV56 and 75-3 were both avirulent on pepper because of the presence of the avirulence gene avrBsT, experiments were designed to test if that avirulence gene caused incompatibility in S. lycopersicoides and the hybrid. Strain 82-8uns containing pST103 (a clone from 75-3 with avrBsT activity) caused HR in S. lycopersicoides and its hybrid with L. esculentum. Strain 82-8uns without the plasmid caused a compatible reaction (Table 6.2). This indicated that avrBsT is responsible for this nonhost HR. Furthermore, the hybrid and the Solanum parent had similar responses to all tested strains. This is evidence that incompatibility originated from the Solanum parent. The possibility of deriving valuable traits from S. lycopersicoides using L. pennellii as a bridging species has been suggested by Rick, et al. (1988).

Possibly, the resistance to XV56 could be transferred to L. esculentum by that method. However, for the immediate need of the resistance to XV56, resistance screening in Lycopersicon species should be conducted.

Screening Lycopersicon genotypes for resistance to XV56

At 24°C, necrosis in leaflets infiltrated with 5 x 10^8 cfu/ml suspensions occurred in BB and H7998 at 72 to 96 hours. Plants of several PI lines developed slight to moderate necrosis 48 hours after infiltration and was considered to be a slow HR. The lines were PI126923, PI126938, Amazon, PI128660, PI159181, PI251306, PI169584, PI195784, PI155371, PI108244, PI155376, PI367982, and PI273015. Four of the PI lines showed moderate to confluent necrosis 48 hours after infiltration. They were PI108245, PI340905, PI155372, and PI79532. Except for PI155372 and PI79532, a degree of rapid necrosis was not observed under the greenhouse conditions of incubation. It is possible that some of these slow HR's are temperature sensitive. A few other lines developed slight to moderate necrosis under greenhouse conditions, such as PI271385, PI127807, PI126932, and PI262173. This might be due to the faster development of susceptibility because no necrosis was observed at 24 C in these four lines. Therefore, PI155372 and PI79532 might be sources of resistance to XV56.

In the assessment of lesion size, 67% of tested lines had similar lesion size generated by the two inoculation methods,

27% had larger lesion size by the dipping method, and 6% had larger lesions by the carborundum method. Small lesions were observed in 10 lines tested by both method. They were PI126428, PI273445, PI99782, PI114490, PI244672, PI324707, PI155372, PI79532, PI128216, and PI306216. The development of smaller lesions in PI155372 and PI79532 may be a response of HR as observed in the infiltration method of inoculation. Among these lines, fewer lesions were observed in the plants of PI114490, PI128216, and PI306216 by the dipping method. Those lines with smaller lesions and fewer lesions may be good sources for resistance to the new race. Field resistance of those lines with possible resistance to XV56 selected from the above tests need to be examined further.

Table 6.1 Classification of groups and races of Xanthomonas campestris pv. vesicatoria and the reactions of strains 75-3 and XV 56 in differential host plants.

Group	Race	Pepper				Tomato	
		ECW	ECW -10R (Bs1)	ECW -20R (Bs2)	ECW -30R (Bs3)	BB	H7998
Pepper	1	+[a]	+	-	-	-	-
Pepper-tomato	2	+	-	-	+	+	-
Pepper-tomato	3	+	+	-	+	+	-
Tomato	1	-	-	-	-	+	-
XV 56	2	-	-	-	-	+	+

[a] '+' : compatible reaction; '-' : incompatible reaction.

Table 6.2 Virulence phenotypes of strains and transconjugants of X. campestris pv. vesicatoria.

	Solanum lycopersicoides	F_1[a]	BB[b]	ECW[b]
E-3	+[c]	+	+	+
75-3	-	-	+	-
XV56	-	-	+	-
82-8uns[d]	+	+	+	+
82-8uns::pST103[d]	-	-	+	-

[a] F_1 plants of the cross between Solanum lycopersicoides and Lycopersicon esculentum (VF36).

[b] Tomato cultivar Bonny Best (BB) was used to indicate the reaction of L. esculentum parent; pepper cultivar Early Calwonder (ECW) was used to indicate the existence of avrBsT.

[c] '-' as incompatible reaction; '+' as compatible reaction.
[d] 82-8uns is a mutant of 82-8 (race 1 of pepper group) which has lost avrBs2 and avrBs3; pST103 is a DNA clone from 75-3 with avrBsT activity.

Table 6.3 Screening for the resistance to XV56 in tomatoes.

No.	Name of Accessions	HR,24°C[a]	HR,GH[b]	Lesion1[b]	Lesion2[b]
1	Burgis Crackproof	+	+	L	.[c]
2	Bulgarian 136-77	+	+	L	.
3	Improved Garden State	+	+	L	.
4	Heinz 1939	+	+	L	.
5	IRAT L3	+	+	S	.
6	Ont. 7615E	+	+	L	.
7	Ont. 7522	+	+	L	.
8	Tiny Tim	+	+	L	.
9	PI 195616	+	+	L	.
10	TK 70-5	+	+	L	.
11	Jubilee	+	+	L	.
12	Ohio MR13	+	+	L	.
13	Ont. 7710	+	+	L	.
14	Oregon 450	+	+	L	.
15	VC 134	+	+	L	.
16	Okitsu Sozai #1	+	+	L	.
17	Porense	+	+	L	.
18	Ohio 7663	+	+	L	.
19	PROC VS28	+	+	L	.
20	PI 91914	+	+	L	.
21	PI 111409	+	+	L	.
22	PI 124235	+	+	L	.
23	PI 126430	+	+	L	.
24	PI 126923	+/-	+	L	.
25	PI 126938	+/-	+	S	.
26	PI 126939	+	+	L	.
28	PI 127801	+	+	L	.
29	PI 224574	+	+	L	.
30	PI 224709	+	+	L	.
31	PI 272736	+	+	L	.
32	PI 306216	+	+	S*	.
33	PI 319895	+	+	L	.
34	PI 330727	+	+	L	.
36	PI 432913	+	+	L	.
37	PI 432949	+	+	L	.
38	PI 432996	+	+	L	.
39	PI 433010	+	+	L	.
40	PI 433011	+	+	L	.
41	PI 127817	+	+	S*	.
42	PI 127818	+	+	S*	.
43	PI 127824	+	+	L	.
44	PI 128650	+	+	L	.
45	PI 134208	+	+	L	.
46	PI 134417	+	+	S*	.
47	PI 155369	+	+	S*	.
48	PI 199380	+	+	L	.

Table 6.3 --continued

No.	Name of Accessions	HR,24°C[a]	HR,GH[b]	Lesion1[b]	Lesion2[b]
49	PI 212408	+	+	L	.
50	PI 215709	+	+	L	.
51	Heinz 603 F9	+	+	L	.
52	1561	+	+	L	.
53	Heinz 1569	+	+	L	.
54	6204 x C111d	+	+	L	.
55	156	+	+	L	.
56	282	+	+	S	.
57	Rehovot 13 P394	+	+	S	.
58	1559	+	+	L	.
59	Cherry Grande	+	+	S	.
60	Roma VF	+	+	L	.
61	D79040	+	+	S	.
62	Saturn	+	+	S	.
63	Venus	+	+	L	.
64	Red Cherry Large	+	+	L	.
65	Early Red Rock	+	+	L	.
66	817	+	+	S	.
67	Scotia	+	+	L	.
68	Amazon	+/-	+	S	.
72	PI 234255	+	+	S	.
73	PI 129129	+	+	L	.
74	PI 102885	+	+	L	.
75	PI 344102	+	+	L	.
76	PI 272664	+	+	S	.
77	PI 195615	+	+	L	.
78	PI 163253	+	+	S	.
79	PI 128660	+/-	+	S	.
80	PI 159181	+/-	+	S	.
81	PI 251306	+/-	+	S	.
82	PI 159007	+	+	L	.
83	PI 390710	+	+	L	.
84	PI 128663	+	+	S	.
85	PI 102719	+	+	S	.
86	PI 128661	+	+	S	.
87	PI 270248	+	+	L	.
88	PI 319894	+	+	S	.
89	PI 126443	+	+	S	.
90	Farthest North PI 370088	+	+	L	.
92	PI 108245	-	+	L	.
93	PI 340905	-	+	S	.
94	PI 169584	+/-	+	L	.
95	PI 155374	+	+	S	.
96	PI 163252	+	+	S	.
97	PI 195784	+/-	+	S	.
98	PI 272736	+	+	L	.

Table 6.3 --continued

No.	Name of Accessions	HR,24°C[a]	HR,GH[b]	Lesion1[b]	Lesion2[b]
99	PI 128888	+	+	L	.
100	PI 155370	+	+	S	.
101	PI 155371	+/-	+	LS	.
102	PI 108244	+/-	+	LS	.
103	PI 155376	+/-	+	S	.
105	PI 118778	+	+	L	.
106	PI 126408	+	+	L	.
107	PI 129062	+	+	L	.
108	PI 270192	+	+	L	.
109	PI 157992	+	+	L	.
110	PI 309909	+	+	S	.
112	PI 309668	+	+	L	.
114	PI 270243	+	+	L	.
115	PI 283935	+	+	S	.
116	PI 127811	+	+	L	.
117	PI 224594	+	+	L	.
118	PI 271385	+	+/-	S	.
120	PI 128608	+	+	L	.
121	PI 272722	+	+	.	.
122	PI 128642	+	+	L	.
123	PI 126437	+	+	S	.
124	PI 109832	+	+	L	.
125	PI 367982	+/-	+	L	.
126	PI 306215	+	+	L	.
127	PI 379054	+	+	L	L
128	PI 372393	+	+	L	L
129	PI 118789	+	+	S	L
130	PI 272763	+	+	S	L
131	PI 273050	+	+	S	L
132	PI 273076	+	+	L	L
133	PI ????30	+	+	L	L
134	PI 290857	+	+	L	L
135	PI 298933	+	+	L	L
136	PI 126920	+	+	L	L
137	PI 159193	+	+	L	L
138	PI 273088	+	+	L	L
139	PI 273103	+	+	L	L
140	PI 115601	+	+	S	L
141	PI 118409	+	+	L	L
142	PI 126428	+	+	S	S
143	PI 127795	+	+	L	L
144	PI 159006	+	+	S	L
145	PI 273445	+	+	S	S
146	PI 273015	+/-	+	L	L
147	PI 270215	+	+	L	L
148	PI 99782	+	+	S	S

Table 6.3 --continued

No.	Name of Accessions	HR,24°C[a]	HR,GH[b]	Lesion1[b]	Lesion2[b]
149	PI 272837	+	+	S	L
150	PI 224574	+	+	L	L
151	PI 155375	+	+	L	L
152	PI 127816	+	+	.	S
153	PI 127808	+	+	L	L
154	PI 127807	+	+/-	L	S
155	PI 126932	+	+/-	S*	L
156	PI 419203	+	+	L	L
157	PI 262173	+	+/-	L	L
158	PI 129112	+	+	L	S
159	PI 117898	+	+	L	L
160	PI 263715	+	+	L	L
161	PI 128593	+	+	L	L
162	PI 92863	+	+	L	L
163	PI 272776	+	+	L	S
164	PI 270419	+	+	LS	L
165	PI 163245	+	+	LS	L
166	PI 155367	+	+	L	L
168	PI 129134	+	+	S	L
169	PI 114490	+	+	S	S (few lesion)
170	PI 163254	+	+	L	L
174	PI 125831	+	+	L	L
175	PI 124582	+	+	L	L
176	PI 102884	+	+	L	L
177	PI 65023	+	+	L	L
178	PI 291334	+	+	L	L
179	PI 273127	+	+	LS	LS
180	PI 273053	+	+	LS	L
181	PI 195785	+	+	L	L
182	PI 376072	+	+	LS	LS
183	PI 273141	+	+	LS	L
184	PI 273073	+	+	S	L
185	PI 272701	+	+	L	L
186	PI 258469	+	+	L	L
187	PI 233930	+	+	L	L
188	PI 169581	+	+	LS	L
189	PI 215709	+	+	LS	L
190	PI 128601	+	+	L	L
191	PI 128611	+	+	LS	L
193	PI 131880	+	+	LS	L
194	PI 159009	+	+	L	L
195	PI 244672	+	+	S	S
196	PI 419145	+	+	L	L
197	PI 273049	+	+	LS	L
198	PI 244956	+	+	L	S

Table 6.3 --continued

No.	Name of Accessions	HR,24°C[a]	HR,GH[b]	Lesion1[b]	Lesion2[b]
199	PI 92861	+	+	L	L
200	PI 92858	+	+	L	L
201	PI 231730	+	+	L	L
202	PI 159198	+	+	S	L
204	PI 169589	+	+	L	L
205	PI 159008	+	+	L	L
206	PI 155373	+	+	L	L
207	PI 129061	+	+	S	L
208	PI 324707	+	+	S	S
209	PI 177007	+	+	L	L
210	PI 114968	+	+	S	LS
211	PI 155378	+	+	L	L
212	PI 155372	-	-	S	S
213	PI 79532	-	-	S	S
215	PI 304252	+	+	L	L
216	PI 272734	+	+	LS	LS
217	PI 155379	+	+	S	L
219	PI 120278	+	+	L	L
220	PI 100697	+	+	LS	LS
221	PI 91909	+	+	L	L
222	PI 379050	+	+	LS	LS
223	PI 195322	+	+	S	LS (few lesion)
224	PI 273085	+	+	L	L
225	PI 273077	+	+	L	LS
226	PI 390731	+	+	S	LS
227	PI 128178	+	+	S	L
228	PI 262995 (Ailsa Craig)	+	+	L	L
229	PI 155368	+	+	L	L
230	PI 128606	+	+	L	LS
231	PI 128216	+	+	S	S (few lesion)
232	PI 114969	+	+	S	LS
233	PI 111409	+	+	L	L
234	PI 379059	+	+	S	L
235	PI 379057	+	+	S	L
236	PI 375937	+	+	LS	LS
237	PI 306216	+	+	S	S (few lesion)

Table 6.3 --continued

[a] 'HR 24°C' referred to HR observed at 24C and 'HR GH' referred to HR observed at greenhouse. '+': compatible reaction; '-': incompatible reaction (confluent necrosis appeared at 48 hr or less than 48 hr after infiltration.); '+/-': moderate necrosis at 48 hr or less than 48 hr.

[b] 'Lesion1' referred to the carborundum inoculation and 'Lesion2' referred to the surfactant inoculation. 'L' : similar or larger lesion size with BB (lesion in BB is larger than H7998); 'LS': lesion size smaller than BB and larger than H7998; 'S' : similar lesion size with H7998; 'S*': similar lesion size with H7998 but with necrotic ring.

[c] '.': no plants were available for testing.

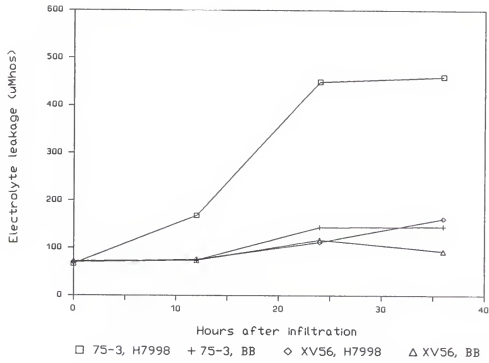

Figure 6.1 Electrolyte leakage in leaflets of Hawaii 7998 (H7998) and Bonny Best (BB) after infiltration with strains of X. campestris pv. vesicatoria.

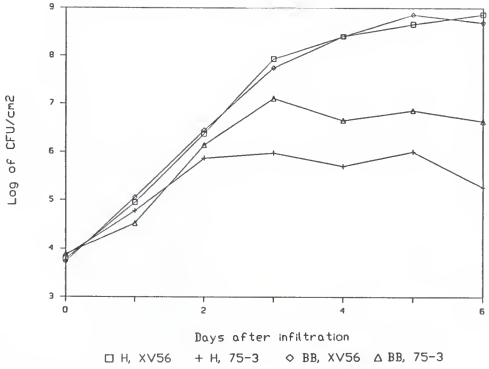

Figure 6.2 Internal bacterial population in Hawaii 7998 (H7998) and Bonny Best (BB) after infiltration with strains of X. campestris pv. vesicatoria.

CHAPTER 7
SUMMARY

1. Hypersensitive resistance in an accession of Lycopersicon esculentum, Hawaii 7998 (H7998) was determined to be temperature sensitive. The incompatible interaction between Xanthomonas campestris pv. vesicatoria (Xcv) and H7998 changed to a compatible reaction based on population dynamics when the incubation temperature increased from 24°C to 30°C.

The F_1 plants of the cross between H7998 and the susceptible L. esculentum breeding line, 7060 displayed an intermediate phenotype based on the time to confluent necrosis after inoculation with Xcv and the internal bacterial population dynamics. Continuous variation of HR development was observed in the F_2 population of this cross. Therefore, the HR seems to be inherited as a quantitative trait.

The possibility of the existence of avirulence genes other than the cloned avrRxv in the strain 90-14 (race 1 of the tomato group of Xcv) was suggested by the dynamics of development of necrosis in the F_2 population. The peak of the percentage of plants that increased in the level of necrosis was smaller and occurred later when a compatible

strain (XV56) containing avrRxv was used as inocula than when 90-14 was used as inocula.

2. The quantitative inheritance of the HR in H7998 was further demonstrated in the cross between H7998 and the wild species, L. pennellii. The phenotype of the development of necrosis in the F_1 plants was intermediate, but much closer to the susceptible L. pennellii. Continuous variation of the development of necrosis was also observed in the F_2 population.

Several linkages between isozyme marker loci and one morphological marker locus and the genes associated with the HR in H7998 were detected in the backcross to the resistant parent. The linkages were with Prx-1, Skdh-1 on chromosome 1, Pgm-2 on chromosome 4, Got-2 on chromosome 7, Pn on chromosome 8, and Sod-1 on chromosome 11.

4. The field resistance of H7998 to Xcv was associated with HR. This was suggested by the significant correlation between the ratings of field resistance and the ratings of HR development and the same linkages, Prx-1 and Skdh-1, detected with both ratings.

5. A hypersensitive reaction was not observed in the plants of H7998 when a strain of Xcv isolated from Brazil (XV56) was used as inocula. Based on the reactions in a

pepper cultivar Early Calwonder (ECW) and a set of near-isogenic lines of ECW containing different resistance genes, XV56 was identified as a strain of the tomato group of Xcv. Since H7998 was susceptible to XV56, it was designated as race 2 of the tomato group.

About 200 tomato accessions were screened for resistance to XV56. A slow HR was observed in plants of PI155372 and PI79532. Fewer lesions and smaller lesions were observed in the plants of PI114490, PI128216, and PI306216.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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